# Inflammation in Prediabetes and Type 2 Diabetes: A Potential Target to Improve Outcomes

by

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## Abstract

**Background**: Inflammation is implicated in the development, progression and long-term complications of type 2 diabetes (T2DM). Hyperglycemia and visceral adipose tissue, which are common in people at risk or diagnosed with T2DM, promote inflammation. Hyperinsulinemia, a compensatory mechanism in response to insulin resistance and a result of some pharmacologic treatments for T2DM, may also be proinflammatory, which has important implications for treatment strategies.

**Purpose**: To further understand the effect that diet and its associated relationship with insulin, has on inflammation and T2DM outcomes.

**Aim 1**: Examines the association between fasting insulin and high sensitivity C-reactive protein (hs-CRP), a marker of inflammation, in adults without diabetes, assessing the potential mediating role of visceral adipose tissue, operationalized as waist circumference. Using a multinomial logistic regression, with hs-CRP divided into three levels of risk and average-risk as the base group, in the reduced adjusted model we found that fasting insulin was associated with high-risk (RRR = 1.07, $p < .001$) and low-risk (RRR = 0.91, $p < .001$) hs-CRP. In the full adjusted model also controlling for waist circumference, the low-risk RRR was 0.97 ($p < .001$) and high-risk RRR was 1.02 ($p = .002$), reflecting the attenuation of 69% and 68% for each group respectively, indicating this relationship may be partially mediated by visceral adipose tissue.

**Aim 2**: Examines the association between diet (net carbohydrates and total sugar) and white blood cell count (WBC), a marker of inflammation, as well as glucose control (glycated hemoglobin [HbA1c]). We found a significant association between net carbohydrate intake and

WBC (β = 0.001, p = .047) but not between total sugar intake and WBC (β = 0.000, p = .838), after adjusting for potential confounders. We did not find a significant association between HbA1c and either net carbohydrate (β = 0.001, p = .311) or total sugar (β = -0.000, p = .815), after adjusting for potential confounders.

**Aim 3**: Examines the effect of a very low-carbohydrate diet (VLC) compared to a Dietary Approaches to Stop Hypertension diet (DASH) on fasting insulin, insulin resistance, and inflammation in overweight or obese adults with prediabetes or T2DM and hypertension over 4 months. Overall, the VLC group experienced greater reductions in fasting insulin compared to the DASH group (-3.86 µU/mL, p = .027) and Homeostatic Model Assessment 2-Insulin Resistance (HOMA2-IR; -0.51, p = .029). However, these effects were no longer significant after a Bonferroni adjustment for multiple comparisons. The VLC group experienced greater reductions in WBC (-0.72 K/uL, p = .004), which was maintained after a Bonferroni adjustment. Reductions in IL-6 approached significance in the DASH vs. VLC group (0.77 pg/mL, p = .070), although this was not significant after the Bonferroni adjustment. Statistically significant differences between diet groups were not observed for the other tested inflammatory markers.

**Conclusions:** This dissertation adds to the evidence that prevention and treatment efforts for T2DM may be improved if inflammation is addressed. These results suggest that treatment approaches that simultaneously decrease insulin levels while achieving glycemic control may provide additional anti-inflammatory effects.

# Chapter One

## Introduction

In the United States, diabetes now affects 34 million adults, and an additional 88 million have prediabetes. The prevalence of diabetes has increased, and current prevention efforts have not reversed the trend (Centers for Disease Control and Prevention, 2017; U.S. Department of Health and Human Services, 2020). Additional research is needed to prevent and treat this chronic and highly prevalent disease.

Diet and lifestyle programs targeted to those with prediabetes can prevent or delay the progression to type 2 diabetes (T2DM) (Knowler et al., 2002). The Diabetes Prevention Program was a three-arm randomized controlled trial that demonstrated it is possible to prevent or delay the onset of diabetes with intensive lifestyle modification with a goal body weight loss of 7% (Knowler et al., 2002).

T2DM is a chronic progressive disease. It is a result of insulin resistance, which results in progressive dysfunction of beta cells and a concurrent continuing decline in endogenous insulin production (Fonseca, 2009). This decline results in an inability to compensate for sustained hyperglycemia and the insulin-resistant state (Fonseca, 2009). Current treatment guidelines for T2DM focus on glycemic control (American Diabetes Association, 2020). The American Diabetes Association (ADA) recommends that an HbA1c of less than 7% be achieved (American Diabetes Association, 2020), while similarly, the American Association of Clinical Endocrinologists (AACE) recommends that an HbA1c of  less than 6.5% be achieved (Garber et al., 2020). Diabetes also increases the risk of many long-term complications including heart

disease, stroke, nephropathy, retinopathy and neuropathy. Current treatment efforts are not adequately preventing these complications. For example, the prevalence of kidney disease was 37% in adults aged 18 years and older in 2013-2016 (U.S. Department of Health and Human Services, 2020). In addition, diabetes was the seventh leading cause of death in 2017 (U.S. Department of Health and Human Services, 2020). Identifying additional treatment strategies that can contribute to halting or reversing this progression may improve the morbidity and mortality for those diagnosed with this disease.

Chronic inflammation appears to be involved in the pathogenesis (Donath & Shoelson, 2011; Rehman et al., 2017), progression (Rehman et al., 2017), and complications (Panee, 2012) of T2DM. Pedersen et al. (2015), recently linked insulin with the development of inflammation. However, current treatment recommendations do not address inflammation, and frequently use medications that increase endogenous insulin production (American Diabetes Association, 2020; Garber et al., 2020). Therefore, one method of improving current prevention and treatment strategies for prediabetes and T2DM may be to address this chronic inflammation by identifying methods that simultaneously decrease glucose levels and inflammation. Inflammatory markers of interest include: interleukin 6 (IL-6), interleukin 8 (IL-8), tumor necrosis factor-alpha (TNF-α), monocyte chemoattractant protein 1 (MCP-1), high-sensitivity C-reactive protein/C-reactive protein (hs-CRP/CRP), and white blood cell count (WBC) at the high end of the normal range.

An additional consideration with current pharmacological treatment strategies is that they are financially burdensome for the individual and for society. Total costs related to diabetes in the United States totaled $327 billion in 2017 (U.S. Department of Health and Human Services, 2020), and the cost of insulin increased 300% between 2002 and 2013 (Kesselheim, Avorn, &

Sarpatwari, 2016). Thus, more efficacious and cost-effective treatment options are imperative to help prevent significant morbidity and mortality for those impacted with this disease.

## Potential for Dietary Interventions

Diet may be one way to decrease chronic inflammation observed in T2DM. Dietary interventions that simultaneously lower insulin and glucose levels may be more effective at decreasing inflammation than solely relying on glycemic control. Therefore, it is justified to examine how diet-associated insulin secretion affects inflammation. Previous research suggests that fructose or dietary sugar (combination of glucose and fructose) may promote inflammation (Aeberli et al., 2011; DiNicolantonio, Mehta, Onkaramurthy, & O'Keefe, 2018). Lowering dietary sugar or fructose intake, or carbohydrates in general, may have beneficial effects on inflammation, particularly for those who are metabolically compromised (Brymora et al., 2012; Hallberg et al., 2018). The ADA does not endorse a specific diet, but rather recommends healthy eating patterns (American Diabetes Association, 2020). The ADA recently added low-carbohydrate diets to the recommended Mediterranean or plant-based diets as examples of healthy eating patterns (American Diabetes Association, 2020). However, the ADA recommends long-term dietary studies with clinical outcomes to assess cardiovascular risk and safety (Evert et al., 2019). Positive effects may be observed for adults with diabetes following a low-carbohydrate ketogenic diet for two years (Athinarayanan et al., 2019). Additionally, the standard-of-care dietary treatment for hypertension is the Dietary Approaches to Stop Hypertension (DASH) diet (Whelton et al., 2017). However, its effects have not been extensively studied in people with prediabetes or T2DM. Therefore, there is a gap in the existing knowledge regarding the best diet to simultaneously reduce inflammation as well as glucose levels, an approach that could improve outcomes for those diagnosed with diabetes.

## Impact on Nursing

In the previously mentioned landmark study, Knowler et al. (2002) demonstrated that it was possible to prevent or delay the progression from prediabetes to T2DM by implementing lifestyle changes. This research is the basis of the National Diabetes Prevention Program (DiBenedetto, Blum, O'Brian, Kolb, & Lipman, 2016), currently led by the CDC (Albright & Gregg, 2013). The Center for Medicare and Medicaid Services recently instituted policies to allow for reimbursement of diabetes prevention within a recognized program (Centers for Medicare & Medicaid Services et al., 2017). In 2012, the CDC provided funding for 30 certified DSME programs to implement the National Diabetes Prevention Program (DiBenedetto et al., 2016). Diabetes educators consist of nurses, dieticians, and pharmacists who obtain certification in Diabetes Self-Management Education (DSME) programs to provide education to those diagnosed with diabetes (DiBenedetto et al., 2016). All the DSME program sites chosen exceeded the 5% patient weight loss goal set by the CDC (DiBenedetto et al., 2016). This is higher than is generally observed in previous intervention programs implemented in community settings (Ely et al., 2017). As this is a vast resource of qualified educators, it would be prudent to utilize this system that is already in place to implement any promising dietary program targeted to people with T2DM.

## Purpose

The purpose of this dissertation is to further understand the effect that diet and its associated reduction of insulin, has on inflammation and diabetes outcomes. To that end, we will use a dietary intervention comparing a very-low-carbohydrate (VLC) diet to a Dietary Approaches to Stop Hypertension (DASH) diet to study the relationship between insulin,

inflammation and metabolic dysfunction. Finally, this research will examine whether a VLC diet, or a DASH diet can decrease inflammation.

## Theoretical Approach to Achieve Behavioral Change

It is important when designing lifestyle programs to consider the sometimes difficult task of behavior change. In order to test the efficacy of various dietary treatment strategies, adherence to the dietary intervention will be necessary. Utilizing theory derived from evidence-based research is one method to design more effective dietary interventions.

One concept that has gained attention in assisting people with lifestyle interventions is mindfulness. Mindfulness may be defined as a state of awareness and attention to present events and experience (Brown, Ryan, & Creswell, 2007). Mindfulness was shown to be an appropriate strategy for assisting participants to make behavior change (Brown et al., 2007). Interestingly, preliminary research suggests that mindfulness has the capacity to decrease inflammation (Brown et al., 2007; Fountain-Zaragoza & Prakash, 2017). Possible mechanisms for the preliminary findings may include effects on the hypothalamic-pituitary-adrenal axis or sympathetic nervous system (Fountain-Zaragoza & Prakash, 2017). Therefore, participant education to increase mindfulness would be an appropriate adjunct to dietary interventions utilized to decrease inflammation.

## Specific Aims and Hypotheses

**Aim 1**: Examine the association between fasting insulin and high-sensitivity C-reactive protein (hs-CRP), a marker of inflammation, using the National Health and Nutrition Examination Survey, 2005-2010. I hypothesized a significant positive association between fasting insulin and hs-CRP.

**Aim 2**: Examine the association between net carbohydrate and sugar intake on white blood cell count at the high end of the normal range (another marker of inflammation), and HbA1c, using the National Health and Nutrition Examination Survey, 2011-2016. I hypothesized a significant positive association between net carbohydrate and sugar intake, and white blood cell count and HbA1c.

**Aim 3**: (a) Compare the effects of a very low-carbohydrate diet (VLC) and a Dietary Approaches to Stop Hypertension diet (DASH) diet, on fasting insulin, insulin resistance, and inflammatory markers. I expect clinically significant improvements in fasting insulin, insulin resistance, and inflammatory markers for participants assigned to a VLC diet, compared to lesser improvements for those assigned to the DASH diet arm based on suggestive published data (Al-Sarraj, Saadi, Calle, Volek, & Fernandez, 2009; Asemi, Samimi, Tabassi, Sabihi, & Esmaillzadeh, 2013; Hallberg et al., 2018; Jonasson, Guldbrand, Lundberg, & Nystrom, 2014; Shirani, Salehi-Abargouei, & Azadbakht, 2013; Steckhan et al., 2016).

(b) Examine whether changes in fasting insulin associated with VLC or DASH diets influence inflammatory markers IL-6, IL-8, TNF-α, MCP-1, hs-CRP, or WBC count. I hypothesized a significant positive association between fasting insulin and dietary effect on inflammation.

The following chapters will cover a review of the literature, three manuscripts, and final conclusions and implications for my dissertation. Chapter Two, *Review of Literature on Hyperinsulinemia and Inflammation in Prediabetes and Type 2 Diabetes***,** provides a review and synthesis of the scientific literature addressing the association of insulin, inflammation, and metabolic disorders including prediabetes and diabetes. Chapter Three, *Association Between Fasting Insulin and High-Sensitivity C-Reactive Protein Among Adults Without Diabetes: NHANES 2005-2010*, addresses Aim 1, Chapter Four, *Association Between Net Carbohydrate*

*and Sugar Intake on Inflammation and Hemoglobin A1c in Adults with Diabetes*, addresses Aim 2, and Chapter Five, *Effects of a Very Low-Carbohydrate Diet Versus a Dietary Approaches to Stop Hypertension Diet on Markers of Inflammation*, addresses Aim 3. Finally, Chapter Six will provide final conclusions, implications for practice, and future research plans.

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# Chapter Two

## Review of Literature on Hyperinsulinemia and Inflammation in Prediabetes and Type 2 Diabetes

The following review will cover the research published to March 2019 on hyperinsulinemia and inflammation in prediabetes or metabolic syndrome, and type 2 diabetes (T2DM). Additional topics covered are the pathophysiologies of hyperinsulinemia, insulin resistance, inflammatory markers, and their relation to weight, visceral adipose tissue, prediabetes, metabolic syndrome, T2DM, and hypertension. The final topic is on the impact of a very low-carbohydrate (VLC) diet, or a Dietary Approaches to Stop Hypertension (DASH) diet, on markers of inflammation and the potential role of insulin in this relationship.

### Obesity

In 2016, 71.2% of adults aged 20 and older were overweight or obese (Centers for Disease Control and Prevention, 2017). This has important consequences for metabolic health. Metabolic dysfunction, particularly insulin resistance, diabetes, and inflammation, is more likely to develop in those who are overweight and obese. The prevalence of diabetes is only 6.2% in underweight or normal weight adults, compared to 20.7% of those who are obese (Mendola, Chen, Gu, Eberhardt, & Saydah, 2018). Visceral adipose tissue is more metabolically active and attracts macrophages, which then secrete proinflammatory cytokines such as IL-1, IL-6, IL-8 and TNF-α (Makki, Froguel, & Wolowczuk, 2013). This resulting chronic inflammation is linked to the pathogenesis of metabolic disease including T2DM (Donath & Shoelson, 2011).

### Insulin Resistance

The main actions of insulin are facilitation of glucose and amino acid uptake; synthesis of fat, glycogen and protein; suppression of triglyceride, glycogen and protein degradation; and increased carbohydrate metabolism at the expense of lipid metabolism (Wilcox, 2005). Insulin resistance refers to a decreased sensitivity of cells to the hormonal actions of insulin (Wilcox, 2005) when higher levels of insulin are observed relative to glucose level (Shanik et al., 2008). Furthermore, insulin resistance is more common in those who are overweight or obese, particularly when weight is gained in the abdomen (Wilcox, 2005). Compensatory increases in insulin typically follow peripheral resistance to insulin action (Wilcox, 2005). Insulin resistance has a circular pathway with hyperinsulinemia, in which high levels of insulin further exacerbate insulin resistance through the down regulation of insulin receptors (Shanik et al., 2008). In addition, resistance to insulin does not develop uniformly throughout the body, which may explain why atherogenic effects are observed prior to metabolic effects such as elevated glucose levels (Madonna & De Caterina, 2012). Insulin resistance does not progress to T2DM in everyone, as some are able to compensate by hyperinsulinemia indefinitely (Tabák, Herder, & Kivimäki, 2012).

The oral disposition index, a product of insulin sensitivity and insulin responses, is a method that may predict who will develop T2DM over a period of 10 years (Utzschneider et al., 2009). It illustrates a hyperbolic pattern between insulin sensitivity and insulin responses in subjects with normal β-cell function (Utzschneider et al., 2009). Previous prospective studies identified a decline in the disposition index prior to the rise in glucose to diabetic levels (Utzschneider et al., 2009).

## Pathology Associated with Hyperinsulinemia

A growing body of research finds that hyperinsulinemia is associated with numerous pathologies including neuropathy (Kim, McLean, Philip, & Feldman, 2011), cancer (Giovannucci, 2007; Micucci, Valli, Matacchione, & Catalano, 2016; Perseghin et al., 2012; Vigneri, Goldfine, & Frittitta, 2016; Wysocki & Wierusz-Wysocka, 2010), stroke (Lindahl et al., 2000) and heart disease (Cabrera De León et al., 2015; Despres et al., 1996; Mitsuhashi et al., 2011). Insulin is necessary for the optimal growth of some cancers in vitro (Vigneri et al., 2016). Medications targeting a reduction in insulin levels, such as metformin, improve cancer outcomes (Becker, Dossus, & Kaaks, 2009; Wysocki & Wierusz-Wysocka, 2010). It is possible that hyperinsulinemia is negatively impacting all of these pathologies through a similar pathway. Additional research is needed to understand the underlying temporal precedence and mechanisms behind the association between insulin and metabolic pathologies.

## Hyperinsulinemia and Obesity

The carbohydrate-insulin model of obesity posits that the high-glycemic dietary load is responsible for obesity (Ludwig & Ebbeling, 2018). It suggests that a diet of highly processed carbohydrates produces hormonal responses that promote weight gain, exacerbate hunger, and lower energy expenditure (Ludwig & Ebbeling, 2018). In a recent study examining weight maintenance following a 12% weight loss, participants were assigned to one of three diets varying in carbohydrate content between 20 and 60%, to maintain weight loss. The sample was stratified by postprandial insulin concentration 30 minutes following a glucose load. There was a positive association between the amount of carbohydrate restriction and energy expenditure. With every 10% decrease in carbohydrate intake, participants expended an additional 52 kcal/day. There was an inverse relationship between postprandial insulin levels and energy expenditure, so that participants in the highest insulin tertile before weight loss had expended an

additional 308 to 478 kcal/day (intention-to-treat versus per protocol) when consuming the low carbohydrate versus the high carbohydrate diet. The increased energy expenditure observed with carbohydrate restriction supports the hypothesis that insulin elevations promote weight gain by suppressing energy expenditure (Ebbeling et al., 2018).

Hyperinsulinemia may be a necessary condition for development of obesity. Genetically modified mice rendered incapable of developing hyperinsulinemia, but maintaining euglycemia, lost the capacity to become obese (Mehran et al., 2012). Additionally, bariatric surgery frequently induces diabetes remission within one week of surgery and before significant weight loss occurs (Kelly et al., 2014). Hyperinsulinemia (based on fasting insulin) resolved within days of surgery, but insulin resistance had not normalized over three months following surgery (Kelly et al., 2014).

## Hyperinsulinemia's Role in Metabolic Disease

Plausible biologic mechanisms for hyperinsulinemia's role in the development of disease may be mediated through its proliferative, proinflammatory, and insulin-resistance promoting activation of Ras (Madonna & De Caterina, 2012). Ras GTPases are a family of proteins involved in regulating molecular signals in eukaryotic cells to maintain homeostasis (Díez, Sánchez-Jiménez, & Ranea, 2011; Mor, Aizman, George, & Kloog, 2011). Ras regulates these signals by acting as a molecular switch for a number of functions, including activating immune cells and phosphoinositide 3-kinases (PI3k). Activated PI3K phosphorylates IkB kinase (IKK). This in turn activates NF-kB, an agent that may impair insulin signaling (Mor et al., 2011).

Insulin also has the capacity to increase production of plasminogen activator inhibitor type 1 (PAI-1), endothelin, and expression of surface adhesion molecules (Madonna & De Caterina, 2012). The increased production of PAI-1 protein can have pathologic consequences

due to its role in thrombosis. PAI-1 circulates in the bloodstream and inhibits serine proteases such as tissue plasminogen activator (t-PA), which is necessary to dissolve fibrin clots. Increased levels of PAI-1 have been associated with venous thrombosis, pulmonary embolism, and atherosclerosis (Schneider & Sobel, 2012). Endothelin is a proinflammatory vasoconstrictive peptide produced by the vascular endothelium (Horinouchi et al., 2016). It has been implicated in the development of insulin resistance in skeletal muscle (Horinouchi et al., 2016).

Interactions between monocytes and endothelial cells are also involved in atherosclerosis (Madonna, Pandolfi, Massaro, Consoli, & De Caterina, 2004). For example, vascular cell adhesion molecule-1 (VCAM-1), expressed in endothelial cells, promotes the attachment of the monocyte to the arterial wall. Hyperinsulinemia promotes the expression of VCAM-1 in vitro. As the expression of this molecule is associated with atherosclerosis, this may help to explain the higher rates of heart disease observed in subjects with metabolic disease before the onset of T2DM (Cabrera De León et al., 2015; Després et al., 1996; Madonna et al., 2004; Mitsuhashi et al., 2011).

## Inflammation

Inflammation is a novel potential target for prevention, treatment, and improvement of diabetes outcomes. The etiology of chronic inflammation likely involves multiple factors related to diet, hyperinsulinemia, stress, and obesity. In addition, a number of inflammatory cytokines have been implicated in the development of chronic inflammation including IL-6, IL-8, TNF-α, MCP-1, hs-CRP, and a WBC count at the higher end of the normal range.

## Proinflammatory Markers

### Interleukin 6

The proinflammatory cytokine IL-6 is predominantly produced by adipocytes, as well as monocytes, endothelial cells, fibroblasts, skeletal muscle and macrophages (Makki et al., 2013; Rehman et al., 2017). Part of its inflammatory action is through controlling differentiation, migration, proliferation, and neutrophilic and β cell apoptosis (Rehman et al., 2017). IL-6 also impairs intracellular signaling through suppressors of cytokine signaling 3 (SOCS-3) and Janus kinases/signal transducers and activators of transcription (JAK/STAT) (Rehman et al., 2017). SOCS-3 proteins are expressed when stimulated by cytokines (Barclay, Anderson, Waters, & Curlewis, 2007). These proteins interact with the JAK/STAT pathway as a negative regulator of gene expression (Barclay et al., 2007). For instance, SOCS-3 acts as a negative regulator of the leptin receptor and thus interferes with leptin's role in energy regulation, energy expenditure, and satiety (Gurzov, Stanley, Pappas, Thomas, & Gough, 2016). In obese individuals these leptin actions become dysregulated due to leptin resistance despite high leptin levels. Hyperleptinemia is implicated in β-cell dysfunction (Gurzov et al., 2016). IL-6 is involved in development of insulin resistance and T2DM (Wang et al., 2013). In part, it does so through impairment and destruction of beta cells and progression to T2DM (Rehman et al., 2017).

**Interleukin 8**

IL-8 is a proinflammatory chemokine secreted by adipocytes, monocytes, macrophages, T-lymphocytes, endothelial and epidermal cells (Cimini et al., 2017). It is involved in the initiation and maintenance of the inflammatory process within the adipose tissue (Cimini et al., 2017). IL-8 levels are higher in diabetic subjects compared with controls and are associated with reduced glycemic control (Cimini et al., 2017).

**Tumor Necrosis Factor-α**

TNF-α is secreted by adipocytes (Rehman & Akash, 2016) and macrophages (Kahn, Hull, & Utzschneider, 2006). Elevated levels of TNF-α in obese subjects alter the action of insulin by activating the Jun NH2-terminal kinase (JNK) and nuclear factor kappa-B (NF-kB) pathways (Rehman & Akash, 2016). The JNK family comprises JNK1, JNK2 and JNK 3, of which JNK1 and JNK2 affect insulin signaling (Pal, Febbraio, & Lancaster, 2016). JNKs are activated in response to stress signals, one of which is TNF-α (Pal et al., 2016). NF-kB is a mediator of inflammation but under normal conditions is inactive in the cytoplasm (Nandipati, Subramanian, & Agrawal, 2017). Activation of JNK and NF-kB results in phosphorylation of insulin receptor substrate-1 (IRS1) at serine 307 which impairs insulin signaling (Pal et al., 2016). The activation of the JNK and NF-kB pathways are associated with the development of insulin resistance (Kaneto, 2005; Nandipati et al., 2017).

**Monocyte Chemoattractant Protein-1**

MCP-1 is a chemokine produced from hypertrophied adipocytes and thought to be responsible for some of the initial macrophage infiltration into adipose tissue (Rehman & Akash, 2016). MCP-1 increases during hyperglycemia and continues to be produced by adipose tissue to act in a proinflammatory capacity (Panee, 2012). Its role in the development of obesity, insulin resistance and diabetes was reviewed by Panee (2012).

**High-Sensitivity C-Reactive Protein**

Hs-CRP is an acute-phase reactant produced in the liver and a sensitive marker of low-grade inflammation (Wang et al., 2013). A meta-analysis completed on prospective studies found a significant association between CRP and risk of T2DM (Wang et al., 2013).

**White Blood Cells**

Higher levels of leukocytes, lymphocytes and neutrophils within the normal range have been observed in obese subjects with insulin resistance when compared to obese subjects without it (Ryder et al., 2014). Lymphocytes are positively correlated with insulin levels (Ryder et al., 2014). Subjects in the upper quartiles of total leukocyte count display higher rates of metabolic syndrome when compared to the lowest quartile (Babio et al., 2013). In the longitudinal PREDIMED study, subjects who developed metabolic syndrome during the follow-up period had significantly higher leukocyte counts at baseline (Babio et al., 2013). Finally, a higher WBC count was found in a systematic review and meta-analysis to be significantly associated with a higher risk of T2DM (Gkrania-Klotsas et al., 2010).

## Inflammation and Insulin

Insulin is associated with the development of inflammation (Pedersen et al., 2015). This was demonstrated by reducing insulin levels by about 40% in obese mice treated with diazoxide or streptozotocin. In spite of no significant changes in body weight, and with free access to water and food (60% of calories from lipids), decreasing insulin levels reduced the number of macrophages and inflammatory markers in the adipose tissue.

Infusion of insulin under euglycemic conditions in lean mice stimulated the production of cytokines in the adipose tissue (Pedersen et al., 2015). Infusion of insulin via osmotic minipumps, shifted the macrophage balance from anti-inflammatory M2 to proinflammatory M1 type (Kumar et al., 2018). The shift in macrophage balance was mediated by inducible nitric oxide synthetase (iNOS) leading to extracellular matrix (ECM) build-up and insulin resistance (Kumar et al., 2018). iNOS is an enzyme that generates nitric oxide from L-arginine, an amino acid (Lirk, Hoffmann, & Rieder, 2002). The ECM is the space outside the cell that undergoes remodeling as a result of injury (Williams, Kang, & Wasserman, 2015). Reducing insulin

decreased adipose tissue inflammation along with improved insulin sensitivity (Kumar et al., 2018). Thus obesity, mediated by insulin, can lead to changes in cellular signaling leading to insulin resistance (Williams et al., 2015).

When insulin was used to treat T2DM, it resulted in weight gain, an influx of macrophages, and inflammation (Jansen et al., 2013). However, this influx of macrophages was independent of weight gain, although the levels of proinflammatory cytokines were higher in those who had gained more than 4% body weight (Jansen et al., 2013).

## Metabolic Syndrome

Diagnostic criteria for metabolic syndrome include dysglycemia, elevated blood pressure, elevated triglyceride levels, low levels of high-density lipoprotein cholesterol and central obesity which place people at increased risk for cardiovascular disease and diabetes (Alberti et al., 2009). The presence of three of these risk factors is required for a diagnosis of metabolic syndrome. International cut-off points were agreed upon for all risk factors with the exception of waist circumference. They are for triglycerides 150 mg/dL or higher, for HDL-C, less than 40 mg/dL for males or less than 50 mg/dL for females, for blood pressure 130 mmHg or higher systolic, or 85 mmHg or higher diastolic, and for fasting glucose 100 mg/dL or higher. Taking medication to reduce levels below the cut-off constitutes a diagnostic risk factor. The cut-off point for waist circumference in the United States is 102 cm or more for males and 88 cm or more for females. Waist circumference cut-off points based on race or ethnicity, rather than gender alone, may more accurately define risk.

## Prediabetes

Prediabetes is a component of metabolic syndrome. A diagnosis of prediabetes is made based on a fasting glucose between 100 and 125 mg/dL, a two-hour blood glucose concentration

between 140 and 199 mg/dL post 75-gram oral glucose tolerance test, or a hemoglobin A1c (HbA1c) between 5.7% and 6.4% (Wilson, 2017). Importantly, only some subjects develop hyperglycemia and progress to T2DM, while others compensate for insulin resistance indefinitely.

In the previously mentioned Diabetes Prevention Program trial, participants were randomized to 850 mg of metformin twice daily, a diet and exercise intervention, or a placebo, with a goal of achieving a 7% weight loss (Knowler et al., 2002). The intensive lifestyle arm included a 16-lesson curriculum that provided education about a low-fat diet, exercise, and behavior modification. Over the average follow-up of 2.8 years, the lifestyle intervention decreased the incidence of diabetes by 58%, and the metformin arm decreased it by 31%, compared to the placebo arm. In addition, weight loss was greatest in the lifestyle arm with 50% of participants losing at least 7% of their body weight at 24 weeks (end of guidance period), although this diminished to 38% of participants at the end of the study (Knowler et al., 2002). This suggests that during this trial, there was some propensity for participants to regain weight. Moreover, weight loss achieved in community-based programs tend to be less than that achieved during research trials (Ali, Echouffo-Tcheugui, & Williamson, 2012).

## Hypertension

Hypertension also is a component of metabolic syndrome. Guidelines were recently revised lowering the diagnostic criteria so that elevated blood pressure (previously termed prehypertension) is defined by systolic between 120 and 129 mmHg and diastolic by less than 80 mm Hg; stage 1 hypertension is defined by 130 -139 mmHg systolic or by diastolic between 80 and 89 mmHg; and stage 2 is defined by systolic of 140 mmHg or greater or by diastolic 90 mmHg or greater (Whelton et al., 2017). The effect of insulin on blood pressure is as yet

uncertain (Brands & Manhiani, 2012). More recent research suggests that hyperinsulinemia may raise blood pressure in the presence of concomitant hyperglycemia (Brands & Manhiani, 2012).

## Type 2 Diabetes

A diagnosis of T2DM is made when fasting glucose has reached 126 mg/dL or higher, HbA1c has risen to 6.5% or higher, or a blood glucose of 200 mg/dL or higher two hours after an oral glucose tolerance test. T2DM develops when the body fails to compensate for insulin resistance (Alejandro, Gregg, Blandino-Rosano, Cras-Méneur, & Bernal-Mizrachi, 2015). The loss of β-cells, and their failure to maintain mass and function, leads to relative insulin deficiency that are the key components to the development of T2DM (Chen, Cohrs, Stertmann, Bozsak, & Speier, 2017). Previous studies suggest that the propensity to develop metabolic disease may start with intrauterine exposure to undernutrition, overnutrition, or metabolic disease (Dabelea et al., 2000; Dabelea et al., 2008; Garofano, Czernichow, & Bréant, 1997; Gluckman, Hanson, Cooper, & Thornburg, 2008; Patti, 2013). No clear genetic determinants of T2DM have been established (Alejandro et al., 2015). However, epigenetic changes may be in part responsible for the dramatic rise in T2DM (Guenard et al., 2013; Patti, 2013). A recent comprehensive review by Alejandro et al. (2015) explains the role of β-cell dysfunction as a primary determinant in the development and progression of T2DM. According to Alejandro, β-cells are susceptible to glucolipotoxicity and proinflammatory cytokines. The chronic exposure to elevated glucose levels, in combination with elevated free fatty acids, both markers of insulin resistance in liver and adipose tissue, are involved in inducing β-cell failure. Endoplasmic reticulum (ER) stress is also involved in β-cell failure and the progression of T2DM. The ER is the site where proinsulin is folded to become the final protein insulin. The ER is sensitive to stressors, such as proinflammatory cytokines, which may lead to the accumulation of unfolded proteins. This sets

off the unfolded protein response (UPR), which while initially protective, upon chronic signaling can result in further β-cell failure through apoptosis. Finally, hyperglycemia may lead to β-cell failure through oxidative stress. Hyperglycemia sets off a cascade of events culminating in an excess of reactive oxygen species in the islet leading to β-cell dysfunction.

## Inflammation and Long-term Complications

Inflammation is involved not only in the pathogenesis of T2DM, but also in its long-term complications. Inflammation likely contributes to the development of complications related to diabetes, and decreasing inflammation may improve long-term outcomes (Panee, 2012). Hyperglycemia may increase the release of MCP-1 leading to the development of nephropathy, retinopathy and insulitis (inflammation of the islets of Langerhans leading to the destruction of beta cells). Inflammation is therefore important to investigate as many individuals with T2DM are suffering from long-term complications. In addition, cytokines may play an important role in inflammation. In the ADVANCE trial, cytokine IL-6 was an independent predictor of macrovascular complications and death in people with T2DM (Lowe et al., 2014). IL-8 also is an independent predictor of all-cause mortality in those with acute coronary syndrome (Cavusoglu et al., 2015). This may have important implications for those diagnosed with T2DM, as they are at increased risk of heart disease.

## Effect of Diet on Insulin, Inflammatory Markers, and Weight

Approaches to decrease inflammation in adults with prediabetes or T2DM have included medications, alterations in diet, and plant extracts or other supplements such as vitamin D (Dutta et al., 2014; Panee, 2012). Diets have shown promise to influence fasting insulin, inflammation and weight loss, although dietary interventions to decrease markers of inflammation have met

with varying levels of success. Therefore, a research focus on identifying the optimal diet may have an important role in halting or reversing the progression of T2DM.

Sugar or carbohydrate restriction in general are frequently used in dietary intervention trials. Trials using low-carbohydrate (LC) or very low-carbohydrate (VLC) diets (Forsythe et al., 2008; Hallberg et al., 2018; Hu et al., 2013; Jonasson, Guldbrand, Lundberg, & Nystrom, 2014) reported greater reductions in inflammatory markers than DASH trials (Asemi, Samimi, Tabassi, Sabihi, & Esmaillzadeh, 2013). To our knowledge, trials to date have not directly compared these two diets in a population with prediabetes or T2DM. Furthermore, while high fructose intake, such as by drinking sugar-sweetened beverages has been associated with metabolic disease (Aeberli et al., 2011; DiNicolantonio, Mehta, Onkaramurthy, & O'Keefe, 2018; Rutledge & Adeli, 2007), few trials have utilized dietary interventions solely based on lowering fructose intake (Brymora et al., 2012; Maier et al., 2011). Furthermore, a reduction of caloric intake is also observed when trying to lower fructose intake (Maier et al., 2011).

A version of carbohydrate modification was observed in the DIETFITS trial, a randomized clinical trial that included 609 overweight or obese adults (BMI 28-40) with diabetes (Gardner et al., 2018; Stanton et al., 2017). This trial compared weight loss associated with a "healthy" (LC) diet or a "healthy" low-fat (LF) diet. This trial also sought to investigate whether insulin levels 30 minutes after a glucose challenge influenced the effect of the diet. Both diets were low in high-glycemic carbohydrates and sugar by maximizing vegetable intake and minimizing intake of added sugars, refined flours, and trans fats. In addition, the emphasis was to focus on nutrient-dense whole foods prepared at home whenever possible. The initial 8-week phase of the diet had participants restrict either fat ( LF arm) or carbohydrate ( LC arm), followed by adding back foods with no set upper limit. Subjects were asked to monitor how it

affected their weight-loss progress and adjust the level of restriction accordingly (maintain, restrict, or increase). Carbohydrate intake was 48% in the LF arm and 30% in the LC arm at 12-months. In this trial, where the instruction was to eliminate sugars and refined carbohydrates in both diets, weight loss did not differ among arms and insulin levels did not alter the effect of diet.

Finally, pharmacotherapy that blocks the activity of specific proinflammatory cytokines offers a unique insight into decreasing inflammation as a treatment approach as the positive effects are not associated with weight loss. Decreasing inflammation produced beneficial effects independently of reduced weight or glucose levels, and therefore is a promising area of research to improve outcomes for individuals with diabetes (Dinarello, Simon, & Van Der Meer, 2012; Fleischman, Shoelson, Bernier, & Goldfine, 2008; Larsen et al., 2007).

**Low-carbohydrate (LC) diets**

LC diet is an umbrella term used to identify all carbohydrate-restricted diets from LC to very-low-carbohydrate (VLC) (also known as ketogenic) diet. The level of non-fiber carbohydrate intake in a LC diet varies from 20-35 grams per day up to 40% of calories from carbohydrate. VLC diets fall at the low end of this spectrum, as carbohydrates are restricted to the point at which the body begins using ketones as a fuel source. VLC diets are generally described as high in fat, moderate in protein, and low in carbohydrates. Previous studies provide empirical support for the use of VLC diets in T2DM by documenting weight loss, lowered glucose, and lower levels of inflammatory markers (Hallberg et al., 2018; Jonasson et al., 2014; Saslow et al., 2014; Saslow et al., 2017; Seshadri et al., 2004). A biological explanation for these beneficial effects in studies using VLC diets in adults with T2DM, is their ability to lower insulin levels and thus access adipose tissue for fuel, where beta-hydroxybutyrate is the byproduct of

hepatic oxidation of free fatty acids (Hallberg et al., 2018). Furthermore, these diets generally recommend moderate protein content, which generally is between 80-120g per day (as recommended by the Institute of Medicine) (Trumbo, Schlicker, Yates, & Poos, 2002), with the remaining calories from fat. Dietary fat will vary greatly based on the phase of the diet (Volek & Phinney, 2011). In the weight-loss phase, body fat provides a source of energy for fuel, and dietary fat may be eaten to satiety (Volek & Phinney, 2011). However, as weight-loss goals are achieved, dietary fat consumption is increased. During the weight-maintenance phase of VLC diet, dietary fat intake comprises approximately 65%-70% of calories (Volek & Phinney, 2011). Meals generally include animal foods, cheeses, eggs, fats (butter, oil), nuts, seeds and low-carbohydrate vegetables.

**Dietary Approaches to Stop Hypertension diet (DASH)**

The DASH diet involves lowering sodium to less than 2300 milligrams per day by focusing on foods high in potassium, magnesium, and calcium. Carbohydrate intake of a DASH diet is typically around 55% of calories while fat intake is limited to 20-30% of calories (Chiu et al., 2016). Furthermore, the diet encourages fruits and non-starchy vegetables, lean meats and fish, whole grains and low-fat dairy while limiting foods high in saturated fat, oil and sugar (Sacks et al., 2009).

**Effects on Insulin of Low-Carbohydrate, Very Low-Carbohydrate and Ketogenic Diets**

Insulin is a necessary hormone for increased metabolism of glucose, removal of glucose from circulation through facilitation of carbohydrate metabolism, stimulation of peripheral glucose uptake, and use of glucose as a substrate for synthesis of carbohydrates and fat. By virtue of their low dietary carbohydrate load, VLC diets are theorized to reduce insulin levels. This has been corroborated in multiple studies implementing carbohydrate restriction from a high of 30%

of calories to levels low enough to result in nutritional ketosis. Participants used in these studies have ranged from healthy to those with T2DM. Regardless of the measures of insulin concentration used, including fasting, 24-hour mean, 12-hour, 4-hour postprandial, or area under the postprandial curve, all studies reported a decrease in insulin level (Bazzano et al., 2014; Boden, Sargrad, Homko, Mozzoli, & Stein, 2005; Essah, Levy, Sistrun, Kelly, & Nestler, 2007; Hallberg et al., 2018; Hayes et al., 2007; Lin & Borer, 2016; Ratliff, Mutungi, Puglisi, Volek, & Fernandez, 2009). This reduction of insulin may increase the anti-inflammatory effects of a diet.

**Effects on Inflammation of Low-Carbohydrate, Very Low-Carbohydrate and Ketogenic Diets**

A VLC diet was found to be effective at decreasing inflammatory markers in several studies (Al-Sarraj, Saadi, Calle, Volek, & Fernandez, 2009; Forsythe et al., 2008; Hallberg et al., 2018; Hu et al., 2013; Jonasson et al., 2014; Seshadri et al., 2004). This effect was associated with a decrease in several inflammatory markers: IL-6 (Forsythe et al., 2008; Hu et al., 2013; Jonasson et al., 2014), IL-8 (Forsythe et al., 2008; Hu et al., 2013), TNF-α (Al-Sarraj et al., 2009; Forsythe et al., 2008; Hu et al., 2013), MCP-1 (Forsythe et al., 2008; Hu et al., 2013), and WBC (Bhanpuri et al., 2018). Forsythe et al. (2008) and Jonasson et al. (2014) observed anti-inflammatory effects independent of weight loss. The association between low-carbohydrate diets and a decrease in CRP among adults with metabolic syndrome was not confirmed in a recent meta-analysis (Steckhan et al., 2016). The negative outcome could be due to the inclusion of only three low-carbohydrate studies which varied in carbohydrate content and limitations in study designs.

**Effects on Weight of Low-Carbohydrate, Very Low-Carbohydrate and Ketogenic Diets**

LC diets appear to be an effective tool for weight loss (Forsythe et al., 2008; Gardner et al., 2007; Hallberg et al., 2018; Meng et al., 2017; Saslow et al., 2014). Possible mechanisms include reduced insulin accompanied with increased energy expenditure, decreased appetite, and preservation of lean mass during weight loss. Weight loss among overweight premenopausal women at 12 months was significantly greater in the Atkins group (the lowest carbohydrate intake diet) when compared to the Zone, Ornish and LEARN diet groups (Gardner et al., 2007). A secondary analysis of these data signaled insulin as critical for the additional weight loss. When comparing the Atkins (low-carbohydrate) diet to the Ornish (very low-fat) diet individuals with the highest fasting insulin levels at baseline experienced greater weight loss when assigned to the low-carbohydrate group, whereas those with the lowest fasting insulin level at baseline lost similar amounts of weight when assigned to either group (Gardner et al., 2008).

LC diets may affect appetite by means of reduced insulin (Alsaadi & Van Vugt, 2015) or increased ketones (Gibson et al., 2015). While insulin inhibits appetite in the postprandial state by acting on appetite regulation centers of the brain, this relationship is blunted in insulin resistance (Alsaadi & Van Vugt, 2015). One well-controlled inpatient trial documented the spontaneous reduction in calories observed with LC diets. Nutrient intake and multiple metabolic measurements were monitored closely for one week prior to admission while following their usual diet. Participants were then admitted to a metabolic ward and fed a LC which limited carbohydrates to approximately 21 grams per day and without restrictions on protein or fat, for two weeks. Caloric intake spontaneously decreased from an average of 3,111 before the diet intervention to 2,164 while following the VLC diet while insulin sensitivity improved by 75% (Boden et al., 2005). VLC or ketogenic diets and very low-calorie diets appear to have appetite suppressing effects due to the production of ketones (Gibson et al., 2015).

Metabolic changes that occur during weight loss lead to increased hunger and decreased energy requirements which can promote weight regain (Melby, Paris, Foright, & Peth, 2017). A recent review on the effects of insulin sensitivity during weight loss and weight regain found that improvements in insulin sensitivity did not predict weight regain (Strohacker, McCaffery, Maclean, & Wing, 2014). However, previous research is inconsistent. Other trials suggest that the choice of diet followed during the weight-loss maintenance period is an essential component of preventing weight regain, particularly for those that are metabolically compromised (Hjorth et al., 2017). In these trials, overweight participants with elevated glucose levels were better able to maintain weight loss when following a low glycemic load or a high fiber diet (Hjorth et al., 2017). Further research is needed to understand appetite regulation and its effect on the likelihood of regaining weight.

It has been hypothesized that LC diets also may allow a greater proportion of weight loss from adipose tissue than from the lean mass. Studies have demonstrated a higher fat-specific weight loss while lowering carbohydrate content and/or glycemic load (Goss et al., 2013; Krieger, Sitren, Daniels, & Langkamp-Henken, 2006; Volek, Quann, & Forsythe, 2010; Volek et al., 2004; Volek et al., 2002). However, a recent position statement by the International Society of Sports Nutrition, finds that a specific macronutrient composition does not provide additional benefit to maintaining lean mass while losing weight (Aragon et al., 2017). Thus, this area remains controversial and requires additional research.

**Effects on Insulin and Inflammation of Dietary Approaches to Stop Hypertension Diet**

Two meta-analyses examined the use of the DASH diet on fasting insulin (Shirani, Salehi-Abargouei, & Azadbakht, 2013), insulin resistance (Shirani et al., 2013), and inflammation (Soltani, Chitsazi, & Salehi-Abargouei, 2018). Shirani et al. (2013) found that a

DASH diet could significantly reduce fasting insulin, but not insulin resistance. Furthermore, the DASH diet reduced CRP, but was only significant when DASH was compared to a usual diet and not to an alternative healthy diet (Soltani, Shirani, Chitsazi, & Salehi-Abargouei, 2016). Therefore, it is unclear how the DASH diet will compare to the positive health effects observed in people following a VLC diet.

### Effects of Weight Loss on Inflammation

Since VLC diets frequently produce weight loss, and weight loss is known to decrease the inflammatory marker CRP (Selvin, Paynter, & Erlinger, 2007), it is important to examine the effect on inflammation independently of weight changes. As metabolically active abdominal fat is responsible for the production of inflammatory cytokines, and it is generally reduced in size on VLC diets (Bazzano et al., 2014; Harvey et al., 2019; Volek et al., 2004; Volek et al., 2002) thus providing a potential biologic mechanism for the reduction of inflammation by VLC diets. However, reduction in some inflammatory markers utilizing a VLC diet appears to be independent of weight loss in some studies (Forsythe et al., 2008; Jonasson et al., 2014).

### Effect of Using Mindfulness in Dietary Interventions

Mindfulness interventions have many positive benefits, which include decreasing inflammation in general (Brown, Ryan, & Creswell, 2007; Fountain-Zaragoza & Prakash, 2017; Malarkey, Jarjoura, & Klatt, 2013) and specific proinflammatory markers NF-kB and CRP, in particular (Black & Slavich, 2016). They also may change eating behavior (Daubenmier et al., 2011; Mason et al., 2016). Therefore, this may be a promising intervention to potentially augment anti-inflammatory effects.

## Treatment Cost

Current treatment approaches generally include medications, diet, and exercise (American Diabetes Association, 2020). Cost may be an important consideration in selection of an optimal treatment approach for diabetic patients to reduce hyperglycemia and inflammation. Among adults in the US, 15.6% have an HbA1c greater than 9%, which is well above the less than 7% recommended by the ADA (Centers for Disease Control and Prevention, 2017). Newer classes of drugs may be prohibitively expensive when generic options are not yet available, and the price increases may leave many Americans unable to afford their medications. In fact, the ADA now recognizes this and recommends that affordability now be considered when choosing a treatment approach (American Diabetes Association, 2020). Therefore, identifying the most effective and less costly dietary approaches may help individuals reduce their reliance on medications.

## Conclusions

The association between hyperinsulinemia and numerous chronic diseases warrants further research on the relationship, mechanism, and temporal reference between these variables. Hyperinsulinemia is difficult to study independently of insulin resistance. One current hypothesis is that the current high-glycemic load of diet may be a primary driver of hyperinsulinemia and subsequent obesity and chronic disease (Ludwig & Ebbeling, 2018). A growing body of research is providing insight into the biologic mechanisms behind hyperinsulinemia's role in the development of chronic disease (Madonna & De Caterina, 2012). Inflammation is an important variable in the pathogenesis (Donath & Shoelson, 2011) and progression (Rehman et al., 2017) of T2DM, as well as its long-term complications (Panee, 2012). While insulin also appears to have an independent role in the development of inflammation (Pedersen et al., 2015), its role in the development and perpetuation of chronic inflammation is not completely understood.

Furthermore, the use of exogenous insulin often confounds research outcomes, as it is often used to achieve glycemic goals, a standard of care in diabetes treatment (American Diabetes Association, 2020).

VLC diets appear to decrease inflammation (Al-Sarraj et al., 2009; Forsythe et al., 2008; Hallberg et al., 2018; Hu et al., 2013; Jonasson et al., 2014; Seshadri et al., 2004). Previous research using VLC diet interventions have frequently compared them to low-fat diets (Forsythe et al., 2008; Hu et al., 2013; Jonasson et al., 2014; Seshadri et al., 2004). Since a DASH diet is frequently recommended by American Diabetes Association and the American Heart Association (American Diabetes Association, 2018; Sacks et al., 2017) as a way of reducing metabolic diseases, it is important to find out how a VLC diet compares to a DASH diet with respect to markers of inflammation. Therefore, this research will seek to fill the knowledge gap by comparing the effects of a VLC diet versus a DASH on fasting insulin and markers of inflammation to see whether there is any relationship between insulin and inflammation.

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# Chapter Three

## Association Between Fasting Insulin and High-Sensitivity C-Reactive Protein Among Adults Without Diabetes: NHANES 2005-2010

Type 2 diabetes (T2DM) affects over 34 million U.S. adults and an additional 88 million U.S. adults are estimated to have prediabetes (U.S. Department of Health and Human Services, 2020). T2DM is characterized by hyperglycemia and insulin resistance, which occurs when cells become less responsive to the glucose-lowering actions of insulin (Wilcox, 2005). Hyperglycemia creates a proinflammatory state through multiple mechanisms including altered function of the innate immune system, decreased vascular dilation and its increased permeability, and increased production of proinflammatory cytokines (Jafar, Edriss, & Nugent, 2016). It is also partly responsible for the long-term complications of T2DM (U.S. Department of Health and Human Services, 2020).

Glycemic control is a focus of current T2DM treatment guidelines (American Diabetes Association, 2020). There are currently several classes of glucose-lowering medications for the treatment of T2DM including biguanides, sulfonylureas, meglitinides, thiazolidinediones (TZD), dipeptidyl peptidase 4 (DPP-4) inhibitors, sodium-glucose cotransporter (SGLT2) inhibitors, and glucagon-like peptide-1 receptor agonists (GLP-1s) (Chaudhury et al., 2017). Glycemic control appears to be more effective at preventing microvascular complications such as retinopathy, nephropathy, and neuropathy than macrovascular outcomes such as cardiovascular disease (Ali et al., 2019).

Insulin resistance develops more frequently in those who are overweight or obese, particularly when weight is gained in the abdomen (Wilcox, 2005). Insulin resistance results in compensatory increases in insulin, leading to hyperinsulinemia which is defined by insulin secretion higher than normal relative to blood glucose (Thomas, Corkey, Istfan, & Apovian, 2019). However, there is debate about the optimal range of insulin, and it varies based on fasting versus postprandial status (Crofts, Zinn, Wheldon, & Schofield, 2015).

There is substantial evidence that inflammation is involved in the development, progression, and long-term complications of T2DM, and that visceral adipose tissue and proinflammatory cytokines play a key role in them (Donath & Shoelson, 2011; Panee, 2012). Visceral adipose tissue is far more detrimental compared to fat in other locations in the development of chronic inflammation. It is metabolically active and attracts macrophages, which then secrete proinflammatory cytokines such as interleukin-1 (IL-1), interleukin-6 (IL-6), interleukin-8 (IL-8) and TNF-α (Makki, Froguel, & Wolowczuk, 2013). Therefore, decreasing inflammation may also be an important treatment goal in the prevention and treatment of T2DM.

Hyperinsulinemia has been associated with inflammation (Pedersen et al., 2015; Pradhan, Cook, Buring, Manson, & Ridker, 2003; Yang, Gerber, & You, 2017) and numerous ill health effects including heart disease (Cabrera De León et al., 2015; Despres et al., 1996; Mitsuhashi et al., 2011) and stroke (Lindahl et al., 2000). However, the role of hyperinsulinemia in the development of inflammation is not well understood. Hyperinsulinemia likely plays a role in weight gain (Ebbeling et al., 2018; Ludwig & Ebbeling, 2018; Mehran et al., 2012), which has proinflammatory effects due to increasing visceral adipose tissue. However, proinflammatory effects have been observed independent of weight (Jansen et al., 2013; Pedersen et al., 2015). Understanding the relationship between insulin and inflammation may have important

implications for the treatment and prevention of T2DM, especially because many of the pharmaceutical treatments for T2DM increase insulin levels through endogenous or exogenous means (American Diabetes Association, 2020). Classes of medications that lower blood glucose but increase insulin levels and exacerbate hyperinsulinemia include exogenous insulin (insulin injections) and insulin secretagogues (sulfonylureas, meglitinides) (Crofts, Zinn, Wheldon, & Schofield, 2016). Other classes of medications including DPP-4s and GLP-1s, increase postprandial insulin levels (Chaudhury et al., 2017). However, GLP-1s exert other positive benefits including decreased glucagon, delayed gastric emptying, weight loss (Chaudhury et al., 2017), and improved insulin sensitivity (Saraiva & Sposito, 2014), which may lower hyperinsulinemia in the long-term. Glucose-lowering medications that may decrease hyperinsulinemia include biguanides (e.g. metformin) which improve insulin sensitivity, inhibit gluconeogenesis (Chaudhury et al., 2017), and up-regulate glucose transporter type 4 (GLUT4) (Crofts et al., 2016). GLUT4 is one of a family of GLUT isoforms and it mediates the uptake of glucose in response to insulin (Karnieli & Armoni, 2008). Additionally, SGLT2s decrease insulin requirements and thus hyperinsulinemia by blocking 90% of glucose reabsorption in the kidneys (Ali et al., 2019; Chaudhury et al., 2017). Finally, while TZDs improve insulin sensitivity and don't increase insulin levels, they do have other side effects that may negate this benefit (Crofts et al., 2016).

High-sensitivity C-reactive protein (hs-CRP) is an acute-phase reactant produced in the liver and a sensitive marker of low-grade inflammation (Wang et al., 2013). Hs-CRP was associated with the markers of insulin resistance and fasting insulin among a nondiabetic population, and the relationship was attenuated after controlling for waist circumference (Meng et al., 2007). A positive association between fasting insulin and hs-CRP was also reported in a

population not taking glucose-lowering medications (Yang et al., 2017) and among nondiabetic females (Pradhan et al., 2003). The present study will extend these findings by utilizing differing methodology, a larger sample, and more recent cycles of NHANES data. Additionally, the relationship between insulin and inflammation has been evaluated in populations with T2DM and hyperglycemia (Khatana, Taveira, Dooley, & Wu, 2010). A direct association between CRP and both insulin use and dose were found in participants with a BMI of less than 30, but not in obese participants (Khatana et al., 2010). The proinflammatory features of hyperglycemia make it difficult to separate the effects of increasing insulin from its glucose-lowering effects in participants with T2DM. Additionally, insulin levels vary with T2DM duration and treatment, partly due to hyperglycemia, beta cell failure, and glucose-lowering medications. For these reasons it is difficult to discern the relationship between insulin and the inflammatory hs-CRP marker in a population with T2DM. It remains unclear how much this association between insulin and inflammation is mediated by visceral adipose tissue. More research is needed to identify the relationship between insulin and inflammation, and to begin to examine possible mediators.

## Objective

The objective of the present study was to examine the independent association between fasting insulin and the inflammatory marker hs-CRP in individuals without diabetes. In addition, we examined the role of visceral adiposity in this relationship.

## Methods

Data on hs-CRP, fasting insulin, obesity-related measurements and other characteristics were collected from the National Health and Nutrition Examination Survey (NHANES) 2005-2010, conducted by the National Center for Health Statistics (NCHS) in the Centers for Disease

Control and Prevention. All the participants signed written consent, and the surveys were approved by the NCHS Institutional Review Board. Interviews and physical examinations were completed on a nationally representative sample of non-institutionalized individuals in the United States. A multi-stage sampling design with weighted domains was used to provide national estimates. Details of the sampling design and guidelines with the NHANES study have been described elsewhere (Zipf et al., 2013).

**Participants**

To address the issues related to insulin and glucose levels in participants with T2DM noted above, our sample included adults aged 20 years and older without a diagnosis of diabetes. While we did not exclude anyone based on an upper age limit, NHANES reports the age of all individuals aged 80 years and older as age 80. This allows them to ensure anonymity of participants due to fewer individuals in higher age groups. To reduce the bias in examining the independent associations between fasting insulin and hs-CRP, and make our analysis consistent with previous NHANES surveys, we excluded individuals younger than 20 years of age, those who have a self-reported diagnosis of diabetes, use glucose-lowering medications, use cholesterol-lowering medications (having an anti-inflammatory effect) (Bu, Griffin, & Lichtman, 2011), or who were pregnant (due to changing physiology and waist circumference in pregnancy). The diabetes questionnaire provided information on the use of glucose-lowering medications or insulin injections. Individuals who self-reported use of these medications or treatments were excluded.

**Measures**

**Hs-CRP.** Detection of hs-CRP was done by latex-enhanced nephelometry, which allowed a conversion of the intensity of reflected light to milligrams of hs-CRP per deciliter

(mg/dL). Results were converted to mg/L to provide consistency with AHA risk categories. Chronic conditions such as arthritis and smoking may raise the levels of hs-CRP.

**Fasting insulin.** Only subjects who fasted overnight and were tested in the morning session were included in the study. Insulin was detected in microunits per milliliter (μU/mL) with the ELISA technique, the technique used by NHANES since 2005.

**Waist circumference.** Waist circumference was measured with a flexible measurement tape in centimeters by trained health technicians. The anatomical landmark was the lateral border of the right ilium. A cosmetic pencil was used to note this landmark just above its uppermost lateral border.

**Physical activity status.** Physical activity status was established using the Global Physical Activity Questionnaire (GPAQ), (Centers for Disease Control and Prevention, 2011). This scale has been validated against accelerometer measurements. There was a moderate correlation between the self-reported questionnaires and accelerometer data with a wide interquartile range (Cleland et al., 2014). To reduce recurring small changes in surveys over the years, select survey questions/variables were dichotomized into subjects who participated in moderate or vigorous physical activity, or reporting no moderate or vigorous physical activity. These variables were then merged to create one dichotomous variable indicating whether the participant self-reported moderate or vigorous physical activity.

**Smoking status.** Smoking status was dichotomized into current smoker or non-smoker. Former smokers were categorized as non-smokers.

**Demographic and socioeconomic factors.** Age in years (analyzed as a continuous variable), race and ethnicity (non-Hispanic White/non-Hispanic Black/Other/Mexican or Hispanic), gender, and poverty-income ratio were obtained for each participant. Poverty-income

ratio is a measure used by NHANES to provide an index of the ratio of family income to poverty. NHANES reports any value greater than 5 as 5, and a higher index indicated higher income.

**Statistical Analyses**

Due to the need to utilize the fasting subsample weight (variable WTSAF2YR in NHANES) with the inclusion of insulin, we were unable to use the GLM model with a gamma distribution to account for the skewed nature of hs-CRP as originally planned. GLM models are very sensitive to sampling weights and resulted in a convergence error. Of note, other approaches of modeling the data were tried including log-transforming hs-CRP and using an ordinal model. Both were deemed inferior to Multinomial Logistic Regression in their ability to model the data. The American Heart Association recommends hs-CRP be used as an independent risk factor for cardiovascular disease, classified as low (< 1 mg/L), average (1 - 3 mg/L), and high (> 3 mg/L) risk (Pearson et al., 2003). Multinomial logistic regression, with hs-CRP categorized by the American Heart Association classification criteria was conducted to estimate the associations and p-values, which I hypothesized would be optimal in the low-risk group and increase incrementally in the average-risk and high-risk categories of hs-CRP. Average risk was used as the basis for comparisons. Summary characteristics of the sample will provide national estimates due to the complex sampling design. Number and proportion (expressed as percent) will be used to describe categorical variables while means and standard errors will be used to describe continuous variables with a normal distribution. Continuous variables with highly skewed variables will use the median and interquartile range to describe the distribution. Summary characteristics of the sample will also be analyzed based on the risk category of hs-CRP.

Two adjusted models were developed. The first or reduced model controlled for age, race, gender, smoking status, physical activity, and poverty-income ratio, but did not control for waist circumference. The second or full model controlled for waist circumference (as an indicator of visceral adipose tissue) and the covariates of the first model. These models tested my hypothesis that there was an independent association between fasting insulin and hs-CRP, and that visceral adipose tissue (as reflected in waist circumference) partially mediated this relationship. Therefore, controlling for waist circumference would reduce or attenuate the association between hs-CRP, a measure of inflammation, and fasting insulin. Margins is a post-estimation option available in Stata to aid in understanding and interpreting regression results. This option was used to provide predicted proportions (displayed as a percent) for each categorical variable within high, average and low-risk hs-CRP categories. Unadjusted estimates were analyzed using multinomial logistic regression for each predictor variable and category of hs-CRP. Regressions will use males, non-Hispanic White race, non or former smoker, and no physical activity as the reference groups. Appropriate fasting sampling weights were used due to the complex sampling design to produce national estimates. All analyses were performed using Stata version 16, College Station, TX.

## Results

The demographic distribution of participants and their summary characteristics based on mean or median are presented in Table 1.1. The final sample included 4,527 adults with a mean age of 43.31 years, 49.03% of them male, 12.53% of Mexican or other Hispanic ethnicity, 70.52% of non-Hispanic White, 10.73% of non-Hispanic Black, and 6.22% who identified as another race or multi-racial. Their mean BMI characterized them as overweight, and their fasting insulin was at the high end of normal relative to their normal blood glucose.

Table 1.2 presents means and proportions for summary characteristics of the sample stratified by the three categories of hs-CRP risk level. Table 1.3 provides the unadjusted multinomial logistic regression results showing the association between each variable and high or low-risk compared to average-risk hs-CRP.

Table 1.4 presents the results of model one or the reduced model displaying the association between fasting insulin and hs-CRP adjusted for age, race, gender, smoking status, physical activity, and poverty-income ratio. Fasting insulin level was significantly associated with high-risk hs-CRP compared to average-risk hs-CRP (Relative risk ratio [RRR] 1.05, $p < .001$). Therefore, a one-unit increase in fasting insulin increased the relative risk of having a high-risk hs-CRP relative to average-risk hs-CRP, by a factor of 1.05. The second comparison from this regression compared low-risk hs-CRP to average-risk hs-CRP (RRR .91, $p < .001$). As fasting insulin increases in this contrast by one unit, the RRR for low-risk hs-CRP relative to average-risk hs-CRP would decrease by a factor of 0.91, if other covariates are held constant. Other covariates were also significant in this model. In this reduced model, comparing high-risk hs-CRP to average-risk hs-CRP, and comparing females to males, the expected difference in hs-CRP was RRR 1.88, $p < .001$. This higher probability of having high-risk hs-CRP among females does not appear to be due to weight, as the mean BMI was lower among females (27.93 $kg/m^2$ , SE 0.18) compared to males (28.09 $kg/m^2$ , SE 0.17). When comparing low-risk hs-CRP to average-risk hs-CRP, and comparing females to males, the expected difference in hs-CRP was RRR .89, $p = .178$. The predicted probabilities for categorical variables are also provided in Table 1.4.

Table 1.5 presents the results of model two, or the full model which controls for waist circumference and the other covariates analyzed in model one. In the full model, there was a

significant association (RRR of 1.01, p = .023) in comparison between high and average hs-CRP risk. Thus, after controlling for waist circumference, this association was attenuated (calculated by percent change in beta coefficients) by 68%. This indicates that some of the association observed between fasting insulin and hs-CRP was due to waist circumference (visceral adipose tissue). Therefore, visceral adipose tissue partially mediated the relationship between fasting insulin and hs-CRP. In addition, in the full model, a significant association was observed when comparing low-risk hs-CRP to average-risk hs-CRP (RRR 0.97, p < .001), which was attenuated by 69% between the full and the reduced model. Sex differences were also significant in this full model. Females had greater probability of high-risk hs-CRP than males (RRR 2.39, p < .001). This relationship was also significant when comparing low-risk to average-risk hs-CRP (RRR .62, p < .001). Table 1.6 also presents the predicted probabilities for this model.

## Discussion

Since inflammation may significantly contribute to insulin resistance and development of T2DM, this study examined the association between fasting insulin and hs-CRP, a marker of inflammation. We conducted the analysis by comparing the levels of hs-CRP that are associated with high risk of cardiovascular disease (> 3 mg/L) with subjects presenting average-risk levels of hs-CRP (1 – 3 mg/L) as well as a comparison of subjects displaying low-risk levels of hs-CRP (< 1 mg/L) with those displaying average risk levels. We found a significant association between fasting insulin and high-risk hs-CRP in comparison to average-risk hs-CRP while controlling for age, race, gender, smoking status, physical activity, and poverty-income ratio in the reduced model, and while also controlling for waist circumference in the full model. The association between fasting insulin and high-risk hs-CRP was stronger in the reduced model and was attenuated by 68% between the reduced and full model. The present results confirm a previous

report of the association between insulin and hs-CRP while controlling for waist circumference (Meng et al., 2007). Both our and Meng et al. (2007) studies support the conclusion that this relationship between fasting insulin and inflammation is partially, but not entirely, mediated by waist circumference used here as a measure of visceral obesity. Additionally, there was a significant association between fasting insulin and low-risk hs-CRP compared to average-risk hs-CRP in both the reduced and full models. This is in line with our hypothesis as it illustrates that those with higher levels of fasting insulin are less likely to have an hs-CRP in the low-risk category. Moreover, our results confirm the results of previous research that females are more likely to have elevated hs-CRP (Khera et al., 2005; Lakoski et al., 2006), a relationship that does not appear to be due to weight.

The association between insulin and inflammation may have implications for treatment of those with T2DM. Current T2DM treatment guidelines recommend specific glycemic targets without consideration for the treatments' effect on insulin. Many glucose-lowering medications increase insulin levels such as sulfonylureas and exogenous insulin, while other therapies decrease insulin requirements such as metformin (Kamenova, 2020) and SGLT-2s (Ali et al., 2019). Current medication guidelines recommend metformin as the first line of treatment (American Diabetes Association, 2020; Garber et al., 2020). Metformin controls blood sugar by decreasing hepatic gluconeogenesis and glucose secretion (Kamenova, 2020). It also decreases insulin resistance and hyperinsulinemia (Kamenova, 2020). Additionally, sodium-glucose cotransporter 2 inhibitors (SGLT-2) or glucagon-like peptide-1 receptor agonists (GLP-1s) are recommended for patients with established or at high risk for atherosclerotic cardiovascular disease (Garber et al., 2020).

Medications such as metformin (Triggle & Ding, 2014) and SGLT2s (Ali et al., 2019) have cardiovascular benefits that are not attributed to the decrease in HbA1c. Controlling HbA1c is more effective at preventing microvascular complications such as nephropathy and retinopathy, than macrovascular outcomes such as cardiovascular disease (Ali et al., 2019). Metformin and SGLT-2s improve many markers beyond HbA1c, including hyperinsulinemia (Ali et al., 2019; Triggle & Ding, 2014; UK Prospective Diabetes Study (UKPDS) Group, 1998). Additionally, GLP-1s improve insulin-sensitivity (Saraiva & Sposito, 2014) which may decrease hyperinsulinemia over time. GLP-1s exhibit anti-inflammatory effects, although the mechanism by which this occurs is still being investigated (Drucker, 2018). If higher insulin levels are associated with higher levels of inflammation, using therapies such as SGLT-2s and GLP-1s that simultaneously decrease insulin and glucose levels, should be recommended for all people with T2DM if additional pharmacotherapy is needed in addition to, or in place of, metformin. Based on the promising effects from medication trials, dietary strategies capable of simultaneously decreasing insulin and glucose levels may have similar positive cardiovascular benefits.

GLP-1s and SGLT-2s may be prohibitively expensive for some individuals and are not available in a generic alternative. Dietary options capable of producing similar effects may be a more economically viable option for some. A study based on results from the National Health Interview Survey, found that almost 20% of adults with diabetes reported at least one cost barrier to receiving glucose-lowering medications (Knight, Probst, Liese, Sercye, & Jones, 2016). As carbohydrates are the primary driver of glucose excursions and the concordant increase in insulin, decreasing the carbohydrate load in the diet, may be one effective method. Although typical treatment goals for T2DM do not currently address inflammation (American Diabetes Association, 2020; Garber et al., 2020), decreasing inflammation may improve long-term

outcomes (Panee, 2012). Future studies are needed to evaluate the effect of different dietary approaches to decrease inflammation.

Limitations of the present study include its cross-sectional nature and consequent inability to determine causality. Additionally, while there was a significant association, due to the small RRR and large sample size this relationship may not be clinically significant. The interplay between insulin and inflammation and between insulin, inflammation, and diabetes is complex and likely influenced by metabolic dysfunction, and therefore, the results of the present study may not generalize to a population of adults with T2DM. Despite this limitation, this study does provide insight into the association between fasting insulin and hs-CRP in adults without diabetes. Additional research is needed to increase our understanding of this relationship.

This study found an association between fasting insulin and hs-CRP that was attenuated while controlling for waist circumference, demonstrating a partial contribution of this variable to this relationship. These results suggest that treatment approaches that simultaneously decrease insulin levels as well as glucose levels may provide additive anti-inflammatory effects, and therefore may improve long-term outcomes for adults with type 2 diabetes.

Table 1.1
Demographic Distribution of Study Participants and Mean Summary Characteristics for Anthropometric and Glycemia Variables Among Adults without Diabetes

| Variable | N=4527 |
|---|---|
| Categorical, N in millions (%) | |
| Sex | |
| Male | 75.43 (49.03) |
| Female | 78.39 (50.97) |
| Race | |
| White | 108.48 (70.52) |
| Mexican/ Hispanic | 19.27 (12.53) |
| Black | 16.50 (10.73) |
| Other/Multi | 9.57 (6.22) |
| Smoking | |
| Non/former Smoker | 117.81 (76.59) |
| Current smoker | 36.01 (23.41) |
| Physical Activity | |
| No | 37.59 (24.44) |
| Yes | 116.23 (75.56) |
| Continuous, mean (SE) or median (IQR) based on distribution | |
| Age years, mean (SE) | 43.31 (0.40) |
| Poverty-Income Ratio, Mean (SE) | 3.06 (0.04) |
| Waist Circumference cm, mean (SE) | 96.08 (0.38) |
| Weight kg, mean (SE) | 80.88 (0.38) |

| | |
|---|---|
| Body Mass Index kg/$m^2$, mean (SE) | 28.01 (0.13) |
| Fasting glucose mg/dL, mean (SE) | 98.79 (0.33) |
| Hemoglobin A1c %, mean (SE) | 5.35 (0.01) |
| Fasting insulin µU/mL, median (IQR) | 9.34 (6.00, 15.14) |
| Hs-CRP mg/L, median (IQR) | 1.5 (0.60, 4.00) |

Abbreviations: White – non-Hispanic White, Mexican/Hispanic – Mexican or other Hispanic ethnicity
Black – non-Hispanic Black, other/multi – other race or Multiracial, SE-standard error
Median and interquartile range (IQR) used for highly right skewed distributions
Note: National estimates based on complex survey design

Table 1.2

Means and Proportions for Summary Characteristics by Category of hs-CRP

| Variable | Low Risk hs-CRP < 1 mg/L[2] N=1574 | Average Risk hs-CRP 1-3 mg/L N=1499 | High Risk hs-CRP > 3 mg/L N=1454 |
|---|---|---|---|
| Categorical, N in millions (%) | | | |
| Sex | | | |
| Male | 28.43 (37.70) | 27.12 (35.96) | 19.87 (26.34) |
| Female | 25.04 (31.95) | 23.82 (30.38) | 29.53 (37.67) |
| Race | | | |
| White | 38.05 (34.77) | 36.20 (33.10) | 34.23 (32.13) |
| Mexican/ Hispanic | 5.74 (29.05) | 6.92 (36.44) | 6.61 (34.50) |
| Black | 5.00 (37.82) | 5.02 (31.36) | 6.48 (30.82) |
| Other /Multi | 4.68 (40.96) | 2.80 (29.59) | 2.09 (29.45) |
| Smoking | | | |
| Non/former Smoker | 41.61 (35.32) | 38.98 (33.09) | 37.22 (31.60) |
| Current Smoker | 11.87 (32.96) | 11.96 (33.21) | 12.18 (33.83) |
| Physical Activity | | | |
| No | 9.80 (26.07) | 12.11 (32.21) | 15.68 (41.72) |
| Yes | 43.68 (37.58) | 38.83 (33.41) | 33.72 (29.01) |
| Continuous, Mean, (SE) | | | |
| Age, years | 40.39 (.56) | 44.66 (.54) | 45.08 (.46) |
| Poverty-Income Ratio | 3.23 (0.06) | 3.07 (0.06) | 2.88 (0.06) |
| Waist Circumference, cm | 87.58 (0.45) | 96.45 (0.55) | 104.90 (0.48) |
| Weight, kg | 72.19 (0.48) | 81.11 (0.65) | 90.07 (0.58) |

| | | | |
|---|---|---|---|
| BMI, $kg/m^2$ | 24.62 (0.16) | 27.89 (0.20) | 31.82 (0.20) |
| Fasting glucose, mg/dL | 96.10 (0.31) | 98.37 (0.45) | 102.14 (0.74) |
| HbA1c% | 5.23 (0.01) | 5.35 (0.01) | 5.49 (0.02) |
| Fasting insulin, μU/mL | 7.90 (0.19) | 11.15 (0.31) | 15.53 (0.35) |

Abbreviations: White – non-Hispanic White, Mexican/Hispanic – Mexican or other Hispanic ethnicity Black – non-Hispanic Black, other/multi – other race or Multiracial, SE – standard error
Note: National estimates based on complex survey design

Table 1.3

Unadjusted Associations Between Covariates and hs-CRP Category using Multinomial Logistic Regression

| Covariate | Comparing Low-Risk to Average-Risk hs-CRP RRR (p-value[1]) | Comparing High-risk to Average-Risk hs-CRP RRR (p-value[2]) |
|---|---|---|
| Age | 0.98 (<.001) * | 1.00 (.503) |
| Sex | | |
| Male | 1.0 (Reference) | 1.0 (Reference) |
| Female | 1.00 (.971) | 1.69 (<.001) * |
| Race | | |
| White | 1.0 (Reference) | 1.0 (Reference) |
| Mexican/Hispanic | 0.79 (.041) * | 1.01 (.918) |
| Black | 0.95 (.622) | 1.36 (.005) * |
| Other/Multi | 1.59 (.015) * | 0.79 (.336) |
| Poverty-income Ratio | 1.06 (.023) * | 0.93 (.003) * |
| Smoking | | |
| Non-smoker | 1.0 (Reference) | 1.0 (Reference) |
| Current smoker | .93 (.468) | 1.07 (.437) |
| Physical Activity | | |
| No | 1.0 (Reference) | 1.0 (Reference) |
| Yes | 1.39 (.004) * | .67 (<.001) |

Abbreviations: White - Non-Hispanic White, Mexican/Hispanic – Mexican or other Hispanic ethnicity, Black – non-Hispanic Black, Other/Multi – other race or Multiracial,
[1]P-value calculated with bivariate multinomial logistic regression comparing low-risk hs-CRP (<1) to average-risk (1-3) hs-CRP with each independent variable separately. Reference is the reference group used in the regression, so p-value not provided
[2]P-value calculated with bivariate multinomial logistic regression comparing high-risk hs-CRP (>3) to average-risk (1-3) hs-CRP with each independent variable separately. Reference is the reference group used in the regression, so p-value not provided
*Significant at p-value of .05
Note: National estimates based on complex survey design

Table 1.4.

Model one or Reduced Model: Adjusted Association Between Fasting Insulin and hs-CRP Category Among Adults Without Diabetes

| Independent Variable | Low Risk hs-CRP < 1 mg/L RRR (p-value) | High Risk hs-CRP >3 mg/L RRR (p-value) | Low-Risk hs-CRP < 1 mg/L[1] Predicted Probability | Average-Risk hs-CRP 1-3 mg/L[1] Predicted Probability | High-Risk hs-CRP >3 mg/L[1] Predicted Probability |
|---|---|---|---|---|---|
| Age | 0.98 (<.001) * | 1.00 (.297) | | | |
| Sex | | | | | |
| Male | 1.0 (Reference) | 1.0 (Reference) | 35.14 | 36.83 | 28.03 |
| Female | .89 (.178) | 1.88 (<.001) * | 29.55 | 30.62 | 39.83 |
| Race | | | | | |
| White | 1.0 (Reference) | 1.0 (Reference) | 32.36 | 33.94 | 33.70 |
| Mexican/ Hispanic | 0.83 (.150) | 0.91 (.419) | 27.98 | 35.99 | 36.03 |
| Black | 1.03 (.793) | 1.18 (.131) | 28.14 | 30.69 | 41.17 |
| Other/ Multi | 1.54 (.021)* | 0.73 (.230) | 46.39 | 30.45 | 23.16 |
| Poverty-Income Ratio | 1.04 (.162) | 0.98 (.311) | | | |
| Smoking Status | | | | | |
| Non-smoker | 1.0 (Reference) | 1.0 (Reference) | 32.61 | 33.68 | 33.72 |
| Current Smoker | 0.74 (.006)* | 1.22 (.029) * | 30.95 | 33.39 | 35.66 |
| Physical Activity | | | | | |
| No | 1.0 (Reference) | 1.0 (Reference) | 23.51 | 32.48 | 44.00 |
| Yes | 1.09 (.413) | 0.78 (.027) * | 35.59 | 34.05 | 30.36 |
| Fasting Insulin, µU/mL | 0.91 (<.001) * | 1.05 (<.001) * | | | |

Abbreviations: White - Non-Hispanic White, Mexican/Hispanic – Mexican or other Hispanic ethnicity, , Black – non-Hispanic Black, Other/Multi – other race or Multiracial; hs-CRP, high-sensitivity C-reactive protein, RRR, relative risk ratio.
Reduced Model: controlling for age, sex, race, socioeconomic status (poverty-income ratio), smoking status, physical activity, and fasting insulin
Multinomial logistic regression used in estimations, reference group is the reference group used for each categorical variable
[1]Probabilities calculated from margins command for categorical variables.
* indicated that the difference was significant at the .05 level.
Note: National estimates based on complex survey design

Table 1.5

Model 2 or Full Model: Adjusted Association Between Fasting Insulin and hs-CRP Category Among Adults Without Diabetes

| Independent Variable | Low Risk hs-CRP < 1 mg/L RRR(p-value) | High Risk hs-CRP >3 mg/L RRR(p-value) | Low-Risk hs-CRP < 1 mg/L[1] Predicted Probability | Average Risk hs-CRP 1-3 mg/L[1] Predicted Probability | High-Risk hs-CRP >3 mg/L[1] Predicted Probability |
|---|---|---|---|---|---|
| Age | 0.99 (<.001)* | 1.00 (.646) | | | . |
| Sex | | | | | |
| Male | 1.0 (Reference) | 1.0 (Reference) | 34.54 | 36.68 | 28.78 |
| Female | 0.62 (<.001) | 2.39 (<.001)* | 28.80 | 30.43 | 40.77 |
| Race | | | | | |
| White | 1.0 (Reference) | 1.0 (Reference) | 31.67 | 33.79 | 34.55 |
| Mexican/ Hispanic | 0.81 (.109) | 1.02 (.851) | 27.79 | 35.88 | 36.34 |
| Black | .98 (.833) | 1.18 (.130) | 27.12 | 30.29 | 42.59 |
| Other/ Multi | 1.24 (.291) | 0.86 (.564) | 45.67 | 30.47 | 23.86 |
| Poverty-Income Ratio | 1.05 (.068) | 0.97 (.328) | | | |
| Smoking Status | | | | | |
| Non-smoker | 1.0 (Reference) | 1.0 (Reference) | 31.97 | 33.55 | 34.48 |
| Current Smoker | 0.68 (.002) | 1.32 (.004)* | 30.17 | 33.13 | 36.70 |
| Physical Activity | | | | | |
| No | 1.0 (Reference) | 1.0 (Reference) | 22.60 | 32.03 | 45.37 |
| Yes | 1.10 (.379) | 0.82 (.067) | 34.92 | 33.99 | 31.09 |
| Waist Circumference, cm | 0.95 (<.001)* | 1.04 (<.001)* | | | |
| Fasting Insulin, µU/mL | .97 (<.001)* | 1.01 (.023)* | | | |

Abbreviations: White – non-Hispanic White, Mexican/Hispanic – Mexican or other Hispanic ethnicity, Black – Non-Hispanic Black, Other/Multi – other race or Multiracial; hs-CRP, high-sensitivity C-reactive protein; RRR, relative risk ratio.

Full Model: controlling for age, sex, race, socioeconomic status (poverty-income ratio), smoking status, physical activity, **waist circumference** and fasting insulin

Multinomial logistic regression used in estimations, reference group is the reference group used for each categorical variable

[1]Probabilities calculated from margins command for categorical variables.

* indicated that the difference was significant at the .05 level.

Note: National estimates based on complex survey design

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# Chapter Four

## Association Between Net Carbohydrate and Sugar Intake on Inflammation and Hemoglobin A1c in Adults with Diabetes

Type 2 diabetes (T2DM) is a highly prevalent chronic condition which affects approximately 34 million Americans (U.S. Department of Health and Human Services, 2020). Many adults fail to meet treatment goals. The American Diabetes Association recommends a hemoglobin A1c (HbA1c) goal of less than 7% (American Diabetes Association, 2020). However, CDC data from 2013-2016 reveal that 50% of people diagnosed with diabetes have an HbA1c above the goal of 7%, and 14.6% had an HbA1c above 9% (U.S. Department of Health and Human Services, 2020). Diabetes places individuals at risk for developing many long-term complications, including cardiovascular disease, retinopathy, nephropathy and neuropathy. Hyperglycemia increases this risk (U.S. Department of Health and Human Services, 2020). Inflammation, although not a current treatment target, is also involved in the development of long-term complications related to diabetes (Donath, 2014; Panee, 2012). Therefore, identifying strategies that simultaneously decrease hyperglycemia as well as inflammation may improve outcomes and the risk of long-term complications in this population.

Dietary strategies may be one way to accomplish this goal. The average adult in the United States consumes 17.6 teaspoons (74 grams) of sugar per day (Bowman et al., 2017). This is well above the recommended limit from the American Heart Association (25 grams of added sugar for females and 36 grams for males per day) (Johnson et al., 2009). The daily sugar intake

limit proposed by the World Health Organization is 10% of calories from free sugar (World Health Organization, 2015). For someone consuming 2,000 calories per day, that would equate to 50 grams of sugar. High sugar intake is associated with diabetes (Malik & Hu, 2015) and heart disease (Yang et al., 2014), likely due, in part, because carbohydrates are the primary macronutrient responsible for postprandial glucose excursions (Evert et al., 2019). Moreover, meals that promote a rapid postprandial rise in glucose generate free radicals resulting in inflammation (O'Keefe, Gheewala, & O'Keefe, 2008), and indeed, fructose or dietary sugar (combination of glucose and fructose) (Aeberli et al., 2011; DiNicolantonio, Mehta, Onkaramurthy, & O'Keefe, 2018), and higher levels of refined carbohydrates in general (O'Keefe et al., 2008) promote inflammation. Current treatment guidelines do not address decreasing chronic inflammation as a goal in the treatment of T2DM (American Diabetes Association, 2020). However, it may be possible to improve outcomes in the treatment of T2DM by examining the impact of carbohydrate intake, irrespective of diet type, on glycemic control and inflammation. Therefore, to address this knowledge gap, it will be useful to increase our understanding of the relationship between sugar and net carbohydrate intake (total carbohydrates – fiber) and their effect on hyperglycemia and inflammation in a population with diabetes. Increasing our understanding of these effects may provide insight into the best dietary options to treat T2DM. The purpose of this study is to examine the association between sugar and net carbohydrate content in the diet, on inflammation and HbA1c.

## Methods

The present study focused on an adult population with diabetes, to discern the association between diet (sugar and net carbohydrate content intake), on inflammation and HbA1c, using National Health and Examination Survey (NHANES) data. NHANES is conducted by the

National Center for Health Statistics in the Centers for Disease Control and Prevention and includes a series of two-year cross-sectional surveys to assess health and nutrition status among non-institutionalized residents of the United States. NHANES used a multi-stage sampling design with weighted domains, to provide national estimates. Details of this study have been described elsewhere (Chen, Clark, Riddles, Mohadjer, & Fakhouri, 2020; U.S. Department of Health and Human Services, 2014). Inclusion criteria were adults aged 20 years of age and older with diabetes. A diagnosis of diabetes was determined by a questionnaire, which asked, "doctor told you have diabetes" or "borderline diabetes" and/or an HbA1c of 6.5 or higher. We used white blood cell count (WBC) at the higher end of the normal range as a marker of inflammation in the 2011-2016 NHANES data. WBC at the higher end of the normal range is used to distinguish its function as a marker of inflammation from its supranormal elevations for numerous reasons including active infections. Exclusion criteria included current pregnancy and predictor variables above the $99^{th}$ percentile. There were substantial outliers for predictor variables including carbohydrate and sugar intake. As our goal was to make inferences about the average adult in the United States, we excluded these outliers so that they do not create undue influence in the analysis. Inflammation and hyperglycemia can be modified by multiple factors. Two separate adjusted models were used to examine effects on WBC. The first examined the association between net carbohydrates and WBC, and the second, the association between total sugar and WBC. Both adjusted models also controlled for age, sex, race/ethnicity, poverty-income ratio (measure of socioeconomic status), smoking status, physical activity, use of cholesterol medication and waist circumference. Additionally, two adjusted models assessed the effect on HbA1c. The first examined the association between net carbohydrate and HbA1c, and the second examined the association between total sugar intake and HbA1c. Both models

controlled for age, sex, race/ethnicity, poverty-income ratio, smoking status, physical activity, and waist circumference.

## Outcome Measures

**Marker of inflammation: White blood cell count (WBC).** We measured WBC count as a component of the complete blood cell count because at the higher end of the normal range, it is a useful indicator of inflammation and is associated with both macro and microvascular complications in people with T2DM (Tong et al., 2004). The specimen was collected using an EDTA blood tube and run on a Coulter DxH 800 (hematological analyzer that provides a full hematological profile which includes a count of WBC). Results were reported in 1000 cells per microliter (1000 cells/μL).

**Glycohemoglobin/Hemoglobin A1c (HbA1c).** Results, reported as a percent, provide the average blood glucose over the previous 120 days.

## Covariate Measures

**Demographics.** Age, race, sex, and poverty-income ratio were obtained for each participant. Poverty-income ratio is an NHANES index of the ratio of family income to poverty, with 5 representing ratios of 5 and above. Additionally, anyone aged 80 years of age or older had their age reported as 80. Therefore, age and poverty-income ratio were not trimmed further to the $99^{th}$ percentile.

**Waist circumference.** Waist circumference was measured in centimeters with a flexible tape measure by trained health technicians. The anatomical landmark was the lateral border of the right ilium and was marked with a cosmetic pencil just above the uppermost lateral border. Because visceral adipose tissue increases inflammation (Makki, Froguel, & Wolowczuk, 2013),

and overweight and obesity impact glucose levels (American Diabetes Association, 2020), waist circumference was included as a covariate in all models (effect on WBC and effect on HbA1c).

**Physical activity status.** Since exercise lowers WBC (Loprinzi & Ramulu, 2013), and glucose levels (Church et al., 2010), physical activity was assessed with the Global Physical Activity Questionnaire (GPAQ) (Centers for Disease Control and Prevention, 2017). This questionnaire was validated against accelerometer measurements (Cleland et al., 2014), and the two measurements were moderately correlated (Cleland et al., 2014). Select survey questions/variables were dichotomized into one of the following: (a) participates in moderate or vigorous physical activity, or (b) no report of moderate or vigorous physical activity to reduce recurrent small changes in the activity questionnaire. These variables were then merged to create one dichotomous variable indicating whether participants self-reported moderate or vigorous physical activity. Exercise was included as a covariate in all models (effect on WBC and effect on HbA1c).

**Smoking status.** As it raises WBC (Higuchi et al., 2016) and glucose levels (Clair, Bitton, Meigs, & Rigotti, 2011), smoking was included as a covariate in all models. Smoking status was dichotomized into current smoker or non-smoker with former smokers categorized as non-smokers.

**Carbohydrate intake.** Twenty-four-hour dietary recalls were assessed in all individuals. This component of the interview is called “What We Eat in America (WWEIA)” (Centers for Disease Control and Prevention, 2018). The data were collected using the Automated Multiple Pass Method (AMP), which were validated for assessments of energy intake in adults (Centers for Disease Control and Prevention, 2018). Two interviews were conducted, once during the physical examination, and a second interview by phone. This information was provided in raw

and aggregate form based on participant interviews. Aggregate data included total carbohydrates, total fiber, and total sugar in grams. This study used total mass of dietary carbohydrate and dietary fiber to calculate net carbohydrate intake (total grams of carbohydrate – fiber). A two-day average was calculated when data were available.

**Use of cholesterol-lowering medication.** As statin medications also work by decreasing inflammation (Bu, Griffin, & Lichtman, 2011), this was used as a covariate in the two models examining the effect on WBC.

### Statistical Analysis

Multiple linear regression was used to analyze the effect of sugar and net carbohydrate on WBC. Waist circumference and net carbohydrate intake were split into quintiles, to analyze potential non-linear relationships. Stata provides a margins option from predictions made from a regression model. Predictive margins are one option available to provide additional detail on relationships in a model including p-values and 95% confidence intervals, that may aid in understanding and interpreting a model. This option was used to predict the WBC value after adjusting for the interaction between quintile of waist circumference and quintile of net carbohydrate as a whole, for males and females separately. To analyze the effect of diet on HbA1c, which has a highly right-skewed distribution, generalized linear models with gamma distribution and identity link function were conducted to estimate the associations. Regressions used males, non-smokers, no physical activity, and not taking cholesterol medications as the reference groups for categorical variables. The appropriate sampling weights were used during the analyses as required when complex sampling designs are used to produce national estimates. All analyses were performed using Stata version 16, College Station, TX.

## Results

Table 2.1 provides the demographic distribution of study participants and mean summary characteristics for key study variables. The final sample included 1,709 participants and was used to produce national estimates. The mean age of the sample was 59.53 years, of which 50.26% were male, 14.63% were Mexican or another Hispanic ethnicity, 63.15% were non-Hispanic White, 13.61% were non-Hispanic Black, 5.11% were Asian, and 3.5% were of another race or identified as more than one race.

Dietary macronutrient intake by sex are presented in Table 2.2. Additionally, the mean caloric intake at the lowest carbohydrate quintile was 1,088 kcal, while the highest quintile was 2,855 kcal. In the lowest quintile of carbohydrate intake, the mean fat intake was 44.6 grams, or 37% of calories from fat. Additionally, mean caloric intake was 1,871 kcal for those who reported following a diet compared to 1,968 kcal for those who did not. The proportion of net carbohydrate and sugar intake as a function of total energy intake was similar in the two sexes.

Unadjusted and adjusted estimates between net carbohydrate intake and WBC are reported in Table 2.3. Unadjusted bivariate estimates found that age ($\beta = -0.009$, $p = .006$), female sex ($\beta = 0.181$, $p = .026$), non-Hispanic Black race ($\beta = -0.525$, $p < .001$), Asian race ($\beta = -0.341$, $p = .029$), Multiracial or another race ($\beta = -0.700$, $p = .007$), current smoker ($\beta = 0.565$, $p < .001$), waist circumference ($\beta = 0.017$, $p < .001$), and net carbohydrates ($\beta = 0.001$, $p = .035$), were all correlated with higher WBC. While the unadjusted bivariate association between net carbohydrate intake and WBC was $\beta = 0.001$, $p = 0.035$, after adjusting for age, sex, race, poverty-income ratio, smoking status, physical activity, taking cholesterol medications, and waist circumference, the association between net carbohydrate intake and WBC was $\beta = 0.001$ and $p = .047$. In the adjusted model, female sex ($\beta = 0.267$, $p = .001$), Non-Hispanic Black race ($\beta = -$

0.637, p < .001), Multiracial or other race (β = -0.889, p = .001), current smoker (β = 0.622, p < .001), and waist circumference (β = 0.019, p < .001) were also significant.

The predicted margins were examined using quintiles for waist circumference and net carbohydrate intake for all individuals, and for men and women separately. The interaction between quintile of waist circumference and quintile of net carbohydrate intake on WBC is depicted for all adults in Figure 2.1, for males only in Figure 2.2, and for females only in Figure 2.3. The lowest quintile of net carbohydrate intake had a mean of 102 grams of net carbohydrate intake per day, while the highest quintile had a mean of 333 grams per day. The lowest quintile of waist circumference had a mean of 88 cm, while the highest was 129 cm. For all individuals, at the highest waist circumference, the lowest quintile of net carbohydrate intake resulted in the lowest mean WBC (mean WBC 7.46, 95% CI 6.97, 7.94). This was significantly different (0.72, p = .023) than the 4$^{th}$ highest quintile of net carbohydrate intake that resulted in the highest mean WBC (8.18, 95% CI 7.78, 8.58). In men, at the highest quintile of waist circumference, the 2$^{nd}$ quintile of net carbohydrate (mean 151 grams) resulted in the lowest mean WBC (7.40, 95% CI 6.97, 7.83). When the relationship was analyzed among females at the highest quintile of waist circumference, the relationship was more complex. The highest quintile with a mean net carbohydrate intake of 335 grams (mean WBC 7.60, 95% CI 6.92, 8.27) and lowest quintile with a mean net carbohydrate intake of 99 grams (mean WBC 7.39, 95% CI 6.80, 7.97) resulted in the lowest WBCs.

Adjusted estimates between total sugar and WBC are reported in Table 2.4 (unadjusted are repeated for comparison purposes). After adjusting for age, sex, race, poverty-income ratio, smoking status, physical activity, taking cholesterol medications, and waist circumference (same confounders as effect of net carbohydrate on WBC) there was no association between total sugar

intake and WBC (β = 0.000, p = .838). In this model, female sex (β = .232, p = .004), non-Hispanic Black race (β = -0.646, p < .001), Multiracial or other race (β = -0.878, p = .002), current smoker (β 0.639, p <.001), and waist circumference (β = 0.019, p < .001) were statistically significant..

The results which include unadjusted and adjusted estimates analyzing the association between net carbohydrate intake and HbA1c are reported in Table 2.5. The unadjusted and adjusted association between total sugar and HbA1c are reported in Table 2.6. After adjusting for confounders, neither total net carbohydrates (β = 0.001, p = .311) nor total sugar (β = -0.000, p = .815), were associated with HbA1c. While examining the adjusted association between net carbohydrate and HbA1c, age (β -0.010, p=.038), female sex (β = -0.246, p =.016), Mexican or other Hispanic ethnicity (β = 0.696, p < .001), Non-Hispanic Black race (β = 0.522, p < .001), Asian race (β = 0.366, p = .007), physical activity (β = -0.304, p =.016), and waist circumference (β = 0.016, p < .001) were all significant. When examining the adjusted association between total sugar and HbA1c, age (β = -0.011, p = .022), female sex (β = -0.275, p = .007), Mexican or other Hispanic ethnicity (β = 0.685, p < .001), non-Hispanic Black race (β = 0.515, p < .001), Asian race (β = 0.349, p = .010), physical activity (β = -0.298, p = .018), and waist circumference (β = 0.016, p < .001) were all significant. Examining the association between covariates and HbA1c found that age (β = -.014, p = .003), female sex (β = -.272, p = .012), Hispanic ethnicity (β = .694, p < .001), non-Hispanic Black race (β = .545, p < .001), physical activity (β = -.309, p = .025), and waist circumference (β = .017, p = .001) were significant.

## Discussion

This study tested the hypothesis that total net carbohydrate and sugar intake by adults with T2DM are associated with inflammation as measured by the upper normal range of WBC

counts and HbA1c. After adjusting for confounders, we found a moderate but significant association between net carbohydrate intake and WBC ($\beta = 0.001$, $p = .047$). However, after adjusting for confounders, no association was found between total sugar intake and WBC, between net carbohydrate intake and HbA1c, or between total sugar intake and HbA1c.

The significance of this study is in its scope. It reveals the association between net carbohydrate load and sugar on WBC and HbA1c in a nationally representative sample of adults with diabetes. Although the cross-sectional design of this study is not capable of proving causality, it does suggest that lower net carbohydrate intake may reduce chronic inflammation. This study suggests that subjects with diabetes have some difficulty processing larger carbohydrate loads. The physiologic basis of this inference is due to higher levels of insulin resistance, which is suggested by their higher waist circumference (Stepien et al., 2011).

The mean caloric intake at the lowest carbohydrate quintile was 1,088 kcal and 2,855 kcal at the highest quintile. As lower calorie diets have also been associated with lower levels of inflammation (Gonzalez, Tobia, Ebersole, & Novak, 2012), this may also be responsible for the observed effect. Total fat and saturated fat were also higher at higher quintiles of carbohydrate intake, indicating those at the highest levels of carbohydrate intake, were also following a high fat diet. The combination of a high-calorie, high-fat, high-carbohydrate diet likely led to the higher levels of inflammation. However, it is important to note that advocates of a lower carbohydrate diet for those with T2DM do not necessarily recommend increasing fat or protein in the diet (Volek & Phinney, 2011). It is recommended to eat a moderate amount of protein and fat until satiation (Volek & Phinney, 2011). Individuals trying to lose weight, would obtain a greater percentage of their energy needs from mobilized body fat, not fat consumption. In the present sample, individuals with the lowest quintile of carbohydrate intake, had an average fat

intake of 37% of calories, which is above what is recommended under typical low-fat diets containing < 30% calories as fat (Tobias et al., 2015). The total number of grams of fat is still substantially lower than those following the high-carbohydrate, high fat diet (44.60 grams versus 109.61 grams of fat). This suggests that increased percentage of calories from fat on lower-carbohydrate diets do not lead to substantially greater intake of calories. Additionally, caloric intake was 1,871 kcal for those who reported following a dietary restriction compared to 1,968 kcal for those who did not. Highly refined carbohydrates may increase feelings of hunger and decrease the length of satiation (Chandler-Laney et al., 2014; Pan & Hu, 2011; Spadaro, Naug, EF, Donner, & Colson, 2015). While it is not possible to examine satiation in this sample, it is one possible explanation for lower caloric intake observed in those following a lower carbohydrate diet.

Lower carbohydrate diets may decrease inflammation (Al-Sarraj, Saadi, Calle, Volek, & Fernandez, 2009; Athinarayanan et al., 2019; Forsythe et al., 2008; Hu et al., 2013; Jonasson, Guldbrand, Lundberg, & Nystrom, 2014; Seshadri et al., 2004). While many randomized trial results were limited by small sample size and short duration (Al-Sarraj et al., 2009; Forsythe et al., 2008; Seshadri et al., 2004), positive anti-inflammatory effects were observed at two years in one trial using a very low-carbohydrate diet (Athinarayanan et al., 2019). Therefore, there is still a lack of consensus about the best diet to follow with a diagnosis of T2DM. Large randomized controlled trials of longer duration are needed to understand the nuances of different diet types.

This study has several limitations. First, its cross-sectional nature precludes the ability to determine cause and effect. Based on these results, we were unable to determine definitively whether there is a causal relationship between diet and changes in inflammation or HbA1c. Second, this is a secondary analysis, in which the participants may have additional diagnoses and

confounding factors that we are unable to control for. Third, this study was limited due to the use of aggregate dietary data. Therefore, we were unable to distinguish between added sugar and naturally occurring sugar, and between whole grains versus highly refined carbohydrates. Additionally, the mean number of carbohydrates was 102 grams in the lowest carbohydrate intake quintile, therefore we are unable to make inferences on very low-carbohydrate diets. Furthermore, dietary data was collected via self-report, which may have led to underreporting. Finally, the effect observed between net carbohydrates and WBC was small with a relatively large sample, and therefore the results may not be clinically significant.

The American Diabetes Association does not recommend one specific diet for subjects with diabetes, nor do they set specific recommendations for carbohydrate intake (American Diabetes Association, 2020). Furthermore, the guidelines for diabetes treatment do not include inflammation (American Diabetes Association, 2020). Identifying dietary strategies capable of simultaneously decreasing glucose levels and inflammation may improve the outcomes and decrease long-term complications for those diagnosed with T2DM. Additional research is needed to improve our understanding on the effects of dietary choices on inflammation, and possible benefits of such diets to individuals with T2DM. However, the results of this trial suggest that lower levels of carbohydrate intake may be beneficial for individuals with T2DM.

In this study, we found a significant association between net carbohydrate intake and WBC concentration at the higher range of normal, which reflects inflammation ($\beta = 0.001$, $p = .035$). This relationship persisted after adjusting for confounders ($\beta = 0.001$, $p = .047$). As carbohydrates are the predominant macronutrient responsible for glucose excursions (Evert et al., 2019b), reducing carbohydrate load may improve glycemic control while simultaneously decreasing inflammation.

Table 2.1

Demographic Distribution of Study Participants and Mean Summary Characteristics of Key Variables for Adults with Diabetes

| Variable | All |
|---|---|
| Sample size, N (unweighted) | 1,709 |
| Sample size (weighted) | 311,425,314 |
| Categorical, N in millions (%) | |
| Sex, % | |
| Male | 9.85 (50.26) |
| Female | 9.75 (49.74) |
| Race, % | |
| Mexican/Hispanic | 2.87 (14.63) |
| White | 12.38 (63.15) |
| Black | 2.67 (13.61) |
| Asian | 1.00 (5.11) |
| Other/Multi | 0.69 (3.50) |
| Smoking | |
| Non-smoker | 16.73 (85.35) |
| Current smoker | 2.87 (14.65) |
| Physical Activity | |
| No | 7.51 (38.3) |
| Yes | 12.10 (61.7) |

| | |
|---|---|
| Glucose-Lowering Medications | |
| Taking Oral | 11.23 (61.71) |
| Taking Insulin | 3.74 (19.12) |
| Continuous, mean (SE) | |
| Age, years | 59.53 (0.44) |
| Poverty-Income Ratio | 2.80 (0.06) |
| Waist Circumference, cm | 109.02 (0.53) |
| BMI $kg/m^2$ | 31.68 (0.24) |
| Fasting insulin, μU/mL | 17.96 (0.52) |
| Fasting glucose, mg/dL | 151.45 (2.38) |
| HbA1c % | 7.14 (0.06) |
| Number of years diagnosed with diabetes | 8.32 (1.12) |
| Total sugar, grams | 91.10 (1.58) |
| Net Carbohydrates, grams | 207.93 (2.60) |

Abbreviations: White – non-Hispanic White, Mexican/Hispanic – Mexican or other Hispanic ethnicity, Black – Non-Hispanic Black, Other/Multi – other race or Multiracial SE – standard error, BMI – body mass index
National estimates based on complex sampling survey design.

Table 2.2

Summary Characteristics of Diet and Weight by Sex

| Diet | Males, mean (SE) | Females, mean (SE) |
|---|---|---|
| Total Calories, kcal | 2187.54 (32.54) | 1680.27 (29.26) |
| Net Carbohydrates, grams | 228.48 (3.62) | 187.16 (3.62) |
| Total Sugar, grams | 97.55 (2.50) | 84.58 (1.95) |
| Total Protein, grams | 89.43 (1.41) | 66.45 (1.13) |
| Total Fat, grams | 88.04 (1.68) | 67.89 (1.48) |
| Weight, kg | 94.50 (1.04) | 84.06 (0.99) |

Abbreviations: SE-standard error
National estimates based on complex survey design

Table 2.3

Unadjusted and Adjusted Estimates of the Association Between Net Carbohydrate Intake and White Blood Cell Count

| Independent Variable | Unadjusted β (p-value) | Adjusted β (p-value) |
|---|---|---|
| Age | -0.009 (.006) * | -0.005 (.146) |
| Sex | | |
| Male | 0.0 (Reference) | 0.0 (Reference) |
| Female | 0.181 (.026) * | 0.267 (.001) * |
| Race | | |
| White | 0.0 (Reference) | 0.0 (Reference) |
| Mexican/ Hispanic | 0.019 (.87) | 0.016 (.896) |
| Black | -0.525 (<.001) * | -0.637(<.001) * |
| Asian | -0.341 (.029) * | 0.003 (.983) |
| Other/Multi | -0.700 (.007) * | -0.889 (.001) * |
| Poverty-Income Ratio | -0.059 (.065) | -0.049 (.086) |
| Smoking | | |
| Non-Smoker | 0.0 (Reference) | 0.0 (Reference) |
| Current smoker | 0.565 (<.001) * | 0.622 (<.001) * |
| Physical Activity | | |
| No | 0.0 (Reference) | 0.0 (Reference) |
| Yes | -0.083 (.378) | 0.002 (.982) |
| Taking Cholesterol Medication | | |
| No | 0.0 (Reference) | 0.0 (Reference) |
| Yes | -0.063 (.563) | -0.067 (.554) |
| Waist Circumference, cm | 0.017 (<.001) * | 0.019 (<.001) * |

| | | |
|---|---|---|
| Total Net Carbohydrates, grams | 0.001 (.035) * | 0.001 (.047) * |

Abbreviations: White – non-Hispanic White, Mexican/Hispanic – Mexican or other Hispanic ethnicity, Black – Non-Hispanic Black, Other/Multi – other race or Multiracial WBC – white blood cell count,
* significant at .05
Unadjusted estimates from bivariate regression
Adjusted estimates controlled for age, sex, race, poverty-income ratio, smoking status, physical activity, use of cholesterol medication and waist circumference
National estimates based on complex survey design

Table 2.4

Unadjusted and Adjusted Estimates on the Association Between Total Sugar Intake and White Blood Cell Count

| Independent Variable | Unadjusted β (p-value) | Adjusted β (p-value) |
|---|---|---|
| Age | -0.009 (.006) * | -0.006 (.086) |
| Sex | | |
| Male | 0.0 (Reference) | 0.0 (Reference) |
| Female | 0.181 (.026) * | 0.232 (.004) * |
| Race | | |
| White | 0.0 (Reference) | 0.0 (Reference) |
| Mexican/ Hispanic | 0.019 (.87) | 0.011 (.929) |
| Black | -0.525 (<.001) * | -0.646 (<.001) * |
| Asian | -0.341 (.029) * | -0.005 (.976) |
| Other/Multi | -0.700 (.007) * | -0.878 (.002) * |
| Poverty-Income Ratio | -0.059 (.065) | -0.048 (.090) |
| Smoking | | |
| Non-Smoker | 0.0 (Reference) | 0.0 (Reference) |
| Current smoker | 0.565 (<.001) * | 0.639 (<.001) * |
| Physical Activity | | |
| No | 0.0 (Reference) | 0.0 (Reference) |
| Yes | -0.083 (.378) | -0.005 (.959) |
| Taking Cholesterol Medication | | |
| No | 0.0 (Reference) | 0.0 (Reference) |
| Yes | -0.063 (.563) | -0.070 (.546) |
| Waist Circumference, cm | 0.017 (<.001) * | 0.019 (<.001) * |
| Total Sugar, grams | 0.001 (.506) | 0.000 (.838) |

Abbreviations: White – non-Hispanic White, Mexican/Hispanic – Mexican or other Hispanic ethnicity, Black – Non-Hispanic Black, Other/Multi – other race or Multiracial WBC – white blood cell count,
* significant at .05
Unadjusted estimates from bivariate regression (unchanged from Table 3, provided for easy reference)
Adjusted estimates controlled for age, sex, race, poverty-income ratio, smoking status, physical activity, use of cholesterol medication and waist circumference
National estimates based on complex survey design

Table 2.5

Unadjusted and Adjusted Estimates for the Association Between Net Carbohydrate Intake and Hemoglobin A1c

| Independent Variable | Unadjusted β (p-value) | Adjusted β (p-value) |
|---|---|---|
| Age | -.014 (.003) * | -.010 (.038) * |
| Sex | | |
| Male | 0.0 (Reference) | 0.0 (Reference) |
| Female | -0.272 (.012) * | -0.246 (.016) * |
| Race | | |
| White | 0.0 (Reference) | 0.0 (Reference) |
| Mexican/Hispanic | 0.694 (<.001) * | 0.696 (<.001) * |
| Black | 0.545 (<.001) * | 0.522 (<.001) * |
| Asian | 0.151 (.206) | 0.366 (.007) * |
| Other/Multi | 0.872 (.043) * | 0.695 (.086) |
| Poverty-Income Ratio | -0.062 (.128) | -0.025 (.486) |
| Smoking | | |
| Non-Smoker | 0.0 (Reference) | 0.0 (Reference) |
| Current smoker | 0.172 (.345) | 0.033 (.809) |
| Physical Activity | | |
| No | 0.0 (Reference) | 0.0 (Reference) |
| Yes | -0.309 (.025) * | -0.304 (.016) * |
| Waist Circumference, cm | 0.017 (.001) * | 0.016 (<.001) * |
| Total Net Carbohydrates, grams | 0.001 (.095) | 0.001 (.311) |

Abbreviations: White – non-Hispanic White, Mexican/Hispanic – Mexican or other Hispanic ethnicity, Black – Non-Hispanic Black, Other/Multi – other race or Multiracial. *p<.05
Unadjusted estimates from bivariate regression
Adjusted estimates controlled for age, sex, race, poverty-income ratio, smoking status, physical activity, and waist circumference
National estimates based on complex survey design

Table 2.6

Unadjusted and Adjusted Estimates for the Association Between Total Sugar Intake and Hemoglobin A1c

| Independent Variable | Unadjusted β (p-value) | Adjusted β (p-value) |
|---|---|---|
| Age | -0.014 (.003) * | -0.011 (.022) * |
| Sex | | |
| Male | 0.0 (Reference) | 0.0 (Reference) |
| Female | -0.272 (.012) * | -0.275 (.007) * |
| Race | | |
| White | 0.0 (Reference) | 0.0 (Reference) |
| Mexican/Hispanic | 0.634 (<.001) * | 0.685 (<.001) * |
| Black | 0.545 (<.001) * | 0.515 (<.001) * |
| Asian | 0.151 (.206) | 0.349 (.010) * |
| Other/Multi | 0.872 (.043) * | 0.698 (.080) |
| Poverty-Income Ratio | -0.062 (.128) | -0.025 (.499) |
| Smoking | | |
| Non-Smoker | 0.0 (Reference) | 0.0 (Reference) |
| Current smoker | 0.172 (.345) | 0.045 (.742) |
| Physical Activity | | |
| No | 0.0 (Reference) | 0.0 (Reference) |
| Yes | -0.309 (.025) * | -0.298 (.018) * |
| Waist Circumference, cm | 0.017 (.001) * | 0.016 (<.001) * |
| Total Sugar, grams | -0.000 (.924) | -0.000 (.815) |

Abbreviations: White – non-Hispanic White, Mexican/Hispanic – Mexican or other Hispanic ethnicity, Black – Non-Hispanic Black, Other/Multi – other race or Multiracial, * p<.05
Unadjusted estimates from bivariate regression (unchanged from Table 5 for easy reference)
Adjusted estimates controlled for age, sex, race, poverty-income ratio, smoking status, physical activity, and waist circumference; National estimates based on complex survey design.

Figure 2.1

Interaction Between Quintile of Net Carbohydrate Intake and Quintile of Waist Circumference on WBC in Adults with Diabetes

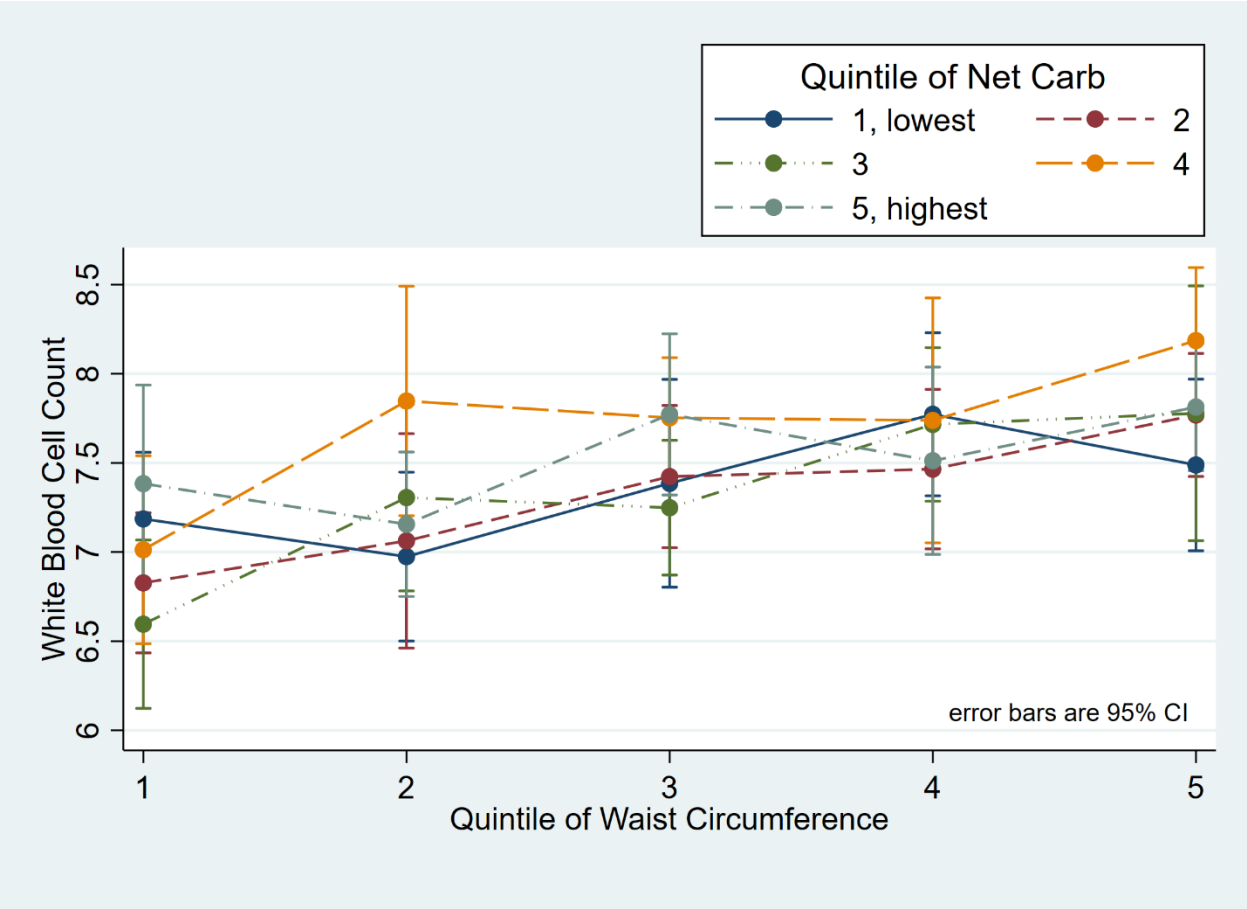


Figure 2.2

Interaction Between Quintile of Net Carbohydrate Intake and Quintile of Waist Circumference on WBC in Males with Diabetes

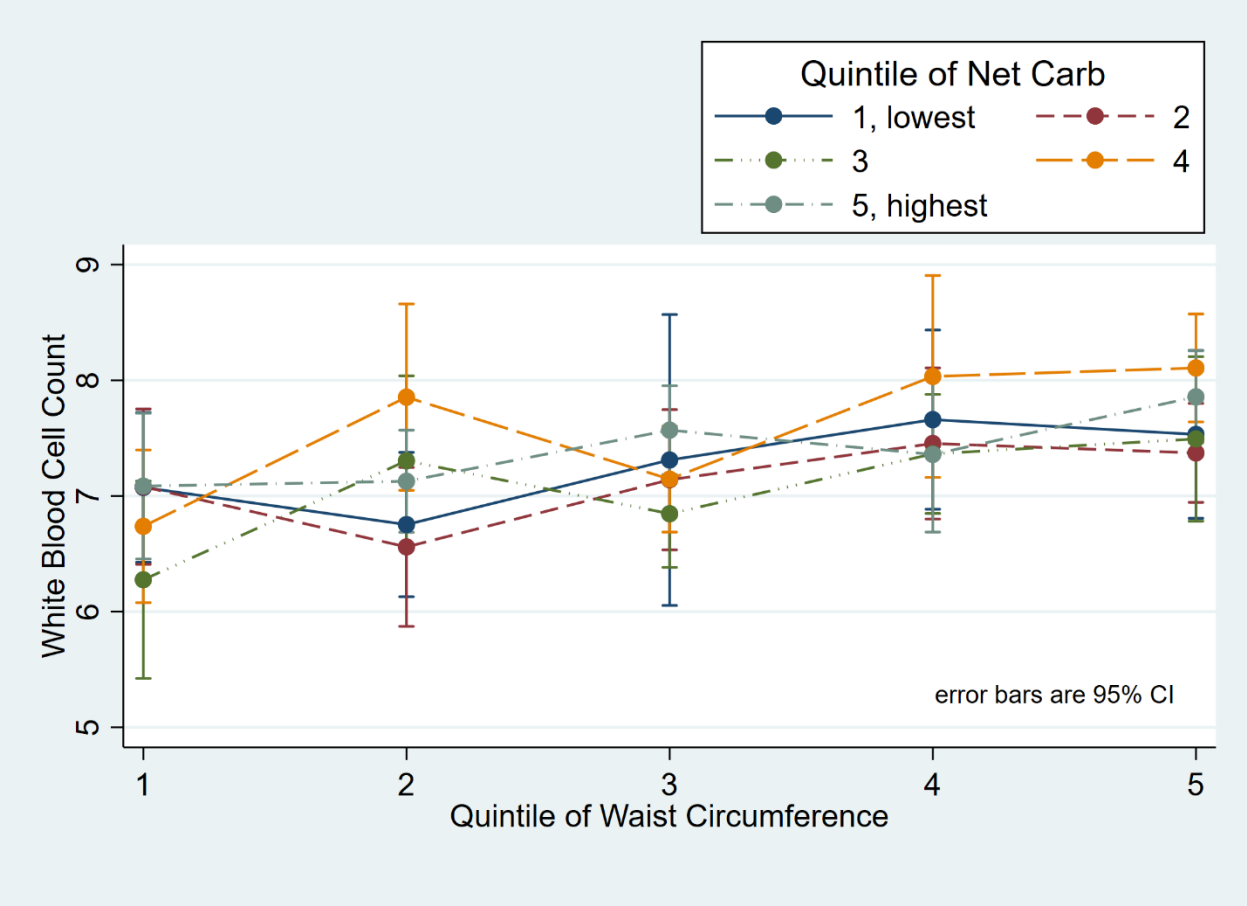
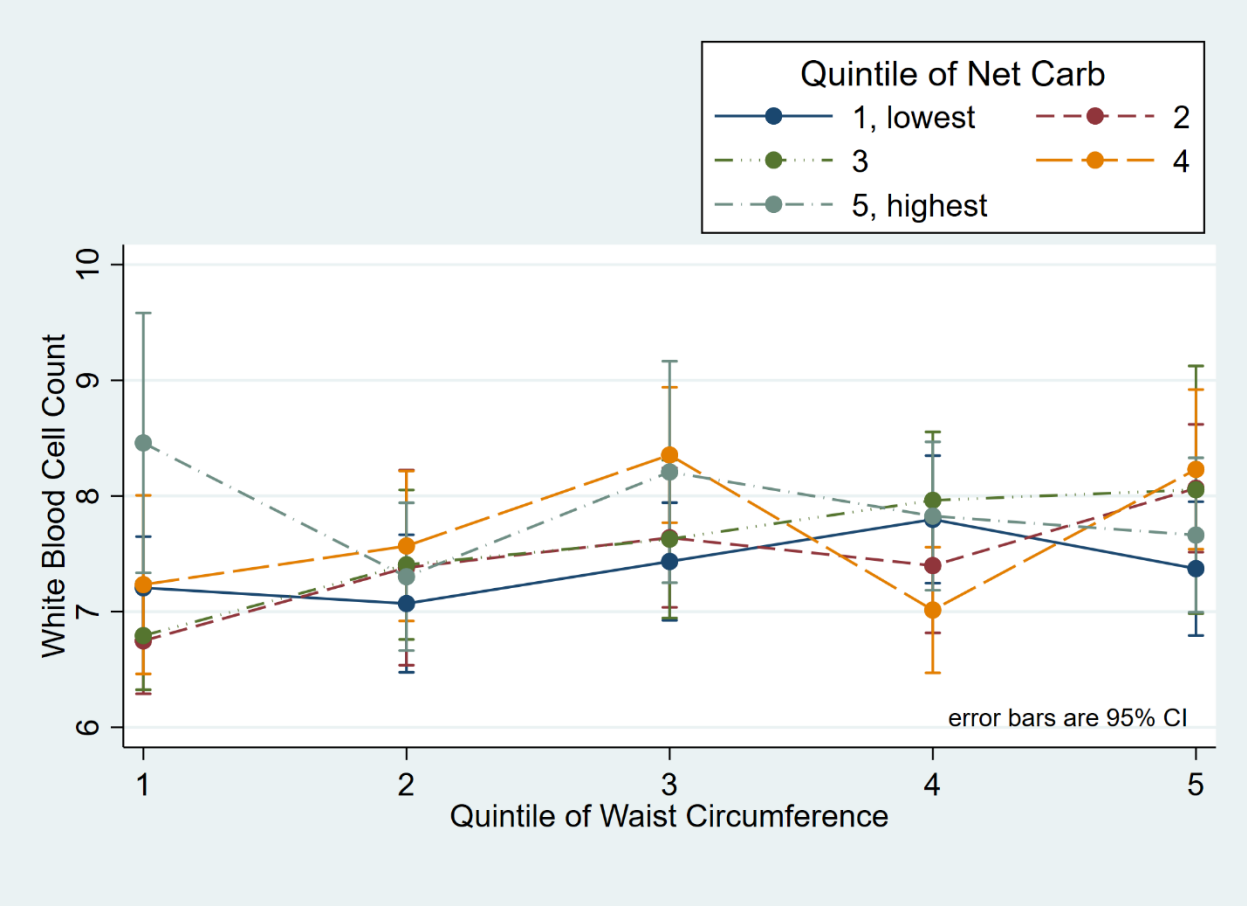


Figure 2.3

Interaction Between Quintile of Net Carbohydrate Intake and Quintile of Waist Circumference on WBC in Females with Diabetes



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# Chapter Five

# Effects of a Very Low-Carbohydrate Diet Versus a Dietary Approaches to Stop Hypertension Diet on Markers of Inflammation

## Introduction

In the United States, the prevalence of diabetes in adults reached 13% in 2018 (U.S. Department of Health and Human Services, 2020). Prediabetes now affects approximately 35% of all adults (U.S. Department of Health and Human Services, 2020). Unfortunately, the prevalence of diabetes appears to be increasing, and current prevention efforts are not reversing the trend (Centers for Disease Control and Prevention, 2017; U.S. Department of Health and Human Services, 2020).

Reducing chronic inflammation may be one method of addressing these increasing trends. Abdominal weight gain is implicated in the development of chronic inflammation. Visceral fat (adipose tissue within the abdominal cavity) is metabolically active and secretes inflammatory cytokines that have been implicated in the pathogenesis, progression and complications of type 2 diabetes (T2DM) (Donath & Shoelson, 2011; Panee, 2012; Rehman et al., 2017). In addition, Pedersen at al. (2015), recently linked increased insulin concentration with the development of inflammation. However, current treatment recommendations do not address inflammation and frequently use medications that increase insulin either by increasing endogenous insulin secretion (e.g. sulfonylureas), or through insulin injections (American Diabetes Association, 2020; Garber et al., 2020). One method of improving current prevention

and treatment strategies may be to address this chronic inflammation by identifying methods that simultaneously decrease glucose levels and inflammation. Diet may be one method of simultaneously decreasing both.

This study supplemented a larger parent study, the Michigan, Hypertension, Diabetes, and Obesity Education Research Online (MHERO) trial, conducted by dissertation committee members Dr. L. Saslow and Dr. C. Richardson, and other co-investigators including a nurse (Dr. L. Jones), a pharmacist (Dr. H. Diez), and a public health researcher (Dr. J. Wolfson).

The purpose of this supplementary study was to further understand the effect that diet, and its associated decrease of insulin, has on inflammation and diabetes outcomes. To that end, we used a dietary intervention comparing a very-low-carbohydrate (VLC) diet to a Dietary Approaches to Stop Hypertension (DASH) diet to study the relationship between insulin, inflammation and metabolic dysfunction. Finally, this research examined whether a VLC diet or a DASH diet decreased inflammation.

## Methods

### Research Design

The present study utilized the protocol of the parent study. The parent study's purpose was to use an interprofessional team to conduct a comparative effectiveness trial to compare a VLC diet to the DASH diet for overweight or obese adults with hypertension and prediabetes or T2DM. The Institute of Medicine defines comparative effectiveness trials as those that generate evidence to compare the benefits and harms of alternative methods to prevent, diagnose, or treat a clinical condition (Institute of Medicine, 2009). MHERO's main aim was to test the feasibility, acceptability, and preliminary comparative efficacy of the diet intervention which will be determined by changes in the primary outcome of systolic blood pressure. Exploratory secondary

outcomes included weight, glycemic control, changes in glucose-lowering medications or antihypertensives, dietary adherence, quality of life, and diabetes-related distress. The parent-study used a 2 x 2 factorial design based on diet type and level of support (standard support versus extra which provided additional education on coping and support strategies).

The present study had a recruitment goal of 120 overweight or obese adults with hypertension and prediabetes or T2DM who would be randomized using a permuted block randomization process, to a 4-month VLC or DASH intervention. Furthermore, participants were stratified by body mass index (BMI; $< 30$ or $\geq 30$) and gender (male or female). BMI and gender were chosen for stratification due to baseline and differential treatment effects that may be observed in these groups (Kennedy et al., 1997; Stommel & Schoenborn, 2010). The study team recruited potentially eligible participants from the University of Michigan health system. Participants received a letter describing the study and received follow-up phone calls.

**Inclusion criteria**

Participants were screened based on whether they met the inclusion criteria from their medical records from the past year or based on their self-report. The inclusion criteria were BMI of 25-50, diagnosis of hypertension (within the past 6 months); current resting systolic blood pressure of 130 mm Hg or higher (safety protocol in place for those with elevated blood pressure of 180 mmHg or higher and experiencing symptoms); diagnosis of either prediabetes or T2DM defined as a hemoglobin A1c (HbA1c) of 5.7% or higher or a two-hour glucose tolerance test greater than 140 mg/dL; aged 18-70 years old; access to the internet and text messaging, ability to engage in light physical activity, sufficient control over their food intake to adhere to study diets; willingness to regularly self-monitor blood pressure, glucose, dietary intake and body weight over the 4-month trial; participation in the trial approved by primary care provider along

with agreement to work with the participant and our research team to manage medication changes.

**Exclusion criteria**

Exclusion criteria included: non-English speaking; current use of insulin, Dilantin, lithium, and warfarin; inability to complete baseline measurements; severe renal or hepatic insufficiency; cardiovascular dysfunction, including diagnosis of congestive heart failure, (angina, arrhythmias, cardiomyopathy, or valvular heart disease); uncontrolled psychiatric disorder; consumption of  greater than 30 alcoholic drinks per week; current chemotherapy treatment; pregnancy or plans to get pregnant in the next 12 months; breastfeeding or less than 6 months postpartum; planned weight-loss surgery or previous bariatric surgery; vegan or vegetarian diet; currently enrolled in a weight-loss program or taking weight-loss supplements and unwilling to stop before enrolling; expecting to move out of the area within 12 months; and any other medical condition that may make either diet dangerous as determined by the study medical team. Due to the number of participants deemed ineligible due to cardiac history, MHERO submitted an IRB amendment to review cardiac history on a case-by-case basis with a study physician.

The study team screened participants to ensure initial eligibility. Eligible participants received a link to complete the informed consent form. Following consent, participants completed a number of steps, including: (1) completing a baseline self-report survey, (2) a letter sent to their primary care provider to secure approval for participation, (3) logging into one online pre-randomization class (4) having their blood pressure, height, weight and waist circumference measured by the study team at the School of Nursing (5) answering two 24-hour food recall phone calls to assess eating habits with a trained dietician at the Michigan Nutrition

Obesity Research Center (6) going to the Michigan Clinical Research Unit (MCRU) to have their blood drawn, and (7) receiving a fibroscan (a noninvasive scan of the liver). Waist circumference measurement and the fibroscan were optional for participants. All other measures were required for enrollment in the study. Enrollment and the intervention were completed on a rolling basis. All outcomes were repeated at 4 months. Participants received their results free of charge.

**Intervention.** MHERO randomized participants to one of four groups. The four groups included DASH diet with extra support (additional education on coping and support strategies), DASH diet with standard support, VLC with extra support and a VLC diet with standard support. MHERO stratified participants by BMI and gender among the four groups. Participants received appropriate links throughout the intervention from a HIPAA-compliant automated system. Weekly emails included coursework, a short survey to assess adherence and any health concerns, short embedded videos and downloadable handouts, links to external resources, as well as transcripts for videos for those participants unable or unwilling to watch.

The VLC diet entailed restricting intake of non-fiber carbohydrates to 20-35 grams per day as such diets have been recommended in T2DM (Hallberg et al., 2018; Jonasson, Guldbrand, Lundberg, & Nystrom, 2014; Saslow et al., 2014; Saslow et al., 2017; Seshadri et al., 2004) for their ability to lower insulin levels and promote oxidation of free fatty acids from the adipose tissue for fuel in the form of beta-hydroxybutyrate (Hallberg et al., 2018). MHERO recommended participants in this diet arm to consume moderate protein between 80-120g (unchanged from prior to enrolling in the intervention unless their intakes were below recommended levels from the Institute of Medicine) (Trumbo, Schlicker, Yates, & Poos, 2002) and to obtain their remaining calories from fat. Foods generally included animal foods, cheeses, eggs, nuts, seeds, low-carbohydrate vegetables and fats. Educational materials encouraged

participants to avoid all trans fats or hydrogenated oils. Materials also recommended avoiding polyunsaturated oils or highly processed oils for cooking due to chemical changes that occur when they are heated past their smoke point. Recommended fats included olive oil, avocado oil, unrefined coconut oil, butter, ghee, animal fats, walnut oil, macadamia nut oil, and limited quantities of sesame-seed oil and flaxseed oil. Additionally, the study team recommended participants to increase magnesium intake if they experienced constipation or post-exercise cramps and to increase salt if they experienced dizziness while standing.

The DASH diet involved lowering sodium to less than 2300 milligrams per day and limiting fat intake to 20-30% of calories. Furthermore, the diet encouraged fruits and vegetables, lean meats and fish, whole grains and low-fat dairy. The diet focused on foods high in potassium, magnesium, and calcium while limiting foods high in saturated fat, oil and sugar (Sacks et al., 2009).

**Extra support.** Half of participants in each diet group received additional education on coping and support strategies which included positive emotions, mindful eating, support for health information seeking and sharing, cooking practice, and behavior.

**Core strategies (Standard Support).** All participants received the core program which encompassed a comprehensive behavior change strategy to encourage and support participants making these changes. Topics and approaches in the program included: goals for the diet, physical activity, and sleep in the form of text messages, mailed materials, and menus. Other components included coaching; social support; dietary self-monitoring using a free online or mobile application; and body weight self-monitoring with a digital scale. Education consisted of 16 online sessions. Education modules contained 5 to 20-minute-long videos and handouts and

links to websites as well. The core strategies used with all participants to assist them in behavior change were as follows:

1. **Goals for diet, physical activity, and sleep.** Participants received goals for diet content and to aim for 7-9 hours of sleep per night. In addition, beginning in week 6, participants received recommendations to engage in moderate-level physical activity for at least 150 minutes per week.

2. **Text messages**. Participants received text messages to assist them in achieving targeted behavior. Messages served as reminders, motivation, and education.

3. **Mailed materials**. Participants received cookbooks at baseline, at one, and at three months to increase participants' self-efficacy for trying and preparing new foods.

4. **Menus**. Participants received written meal plans, and grocery lists.

5. **Coaching**. Coaches were available for participant questions primarily via email, but at times also by phone.

6. **Social support.** MHERO encouraged participation in existing online support groups. The use of existing groups was chosen so that participants could continue if desired once the study was complete.

7. **Dietary self-monitoring using a free online or mobile application**. MHERO asked participants to track their dietary intake with MyFitnessPal.

8. **Body weight self-monitoring using a digital scale**. MHERO asked participants to track their body weight with the digital cellularly-linked scale provided to them to upload their data.

**Safety and consultations with study experts**. Because the intervention, if successful, could have lowered blood pressure and blood glucose, we required monitoring of blood pressure and glucose levels, especially for those at highest risk (those taking antihypertensives or glucose-

lowering medications other than metformin). To that end, participants were provided a home blood pressure monitor and a home glucometer if they were on glucose-lowering medications other than metformin. Participants received information on when to contact their primary care provider as the system was not intended for urgent matters or to replace their primary care provider. During weekly videos participants were asked about symptoms and the previous weeks' blood pressure and glucose levels. Study staff reviewed responses and sent MyChart messages to providers as needed.

**Measures**

This study utilized the following measurements from the parent study: fasting insulin and glucose (for Homeostatic Model Assessment 2 – Insulin Resistance [HOMA2-IR] calculation), height, weight and demographics.

The present supplemental trial added the measurement of the following biomarkers: interleukin 6 (IL-6), interleukin 8 (IL-8), tumor necrosis factor-alpha (TNF-α), monocyte chemoattractant protein 1 (MCP-1), high-sensitivity C-Reactive Protein (hs-CRP), and white blood cell count (WBC). While these are all non-specific biomarkers, they were selected due to their proinflammatory role in the development, progression and long-term complications of T2DM (Jagannathan-Bogdan et al., 2011; Lowe et al., 2014; Panee, 2012; Rehman & Akash, 2016; Rehman et al., 2017; Twig et al., 2013; Wang et al., 2013) and were central to the hypothesis that they will be reduced following the diet intervention.

**HOMA2-IR.** HOMA2-IR was calculated from glucose in mmol/L and fasting insulin in pmol/L using the HOMA2 calculator available from the Diabetes Trials Unit (Diabetes Trials Unit, n.d.). The original HOMA provided an estimate of insulin resistance based on fasting plasma glucose and insulin. HOMA2 accounts for additional variations to provide a more

accurate estimate including hepatic and peripheral glucose resistance, insulin secretion at glucose concentrations above 180 mg/dL, and the effects of proinsulin. The use of the calculator over the formula available to calculate HOMA-IR, provides model-derived estimates versus the linear approximations from the formula (Diabetes Trials Unit, n.d.).

**Pro-inflammatory markers.**

***IL-6.*** IL-6 is involved in development of insulin resistance and T2DM, in part by destruction of beta cells (Rehman et al., 2017). It is predominantly produced by adipocytes, but also by monocytes, endothelial cells, fibroblasts, skeletal muscle and macrophages (Makki, Froguel, & Wolowczuk, 2013; Rehman et al., 2017). Because IL-6 is also produced in skeletal muscle, it is released during exercise where it increases glucose uptake and has an anti-inflammatory effect (Makki et al., 2013). Increased levels of IL-6 are also associated with sleep disturbance (Irwin, Olmstead, & Carroll, 2016).

***IL-8.*** IL-8 is a proinflammatory chemokine secreted by adipocytes, monocytes, macrophages, T-lymphocytes, endothelial and epidermal cells (Cimini et al., 2017). Higher levels of IL-8 are observed in diabetic subjects and are associated with reduced glycemic control (Cimini et al., 2017).

***TNF-α.*** TNF-α is secreted by adipocytes (Rehman & Akash, 2016) and macrophages (Kahn, Hull, & Utzschneider, 2006). It is implicated in the development of insulin resistance and alters the action of insulin in animals (Rehman & Akash, 2016).

***MCP-1.*** MCP-1 is a chemokine produced from hypertrophied adipocytes and thought to be responsible for some of the initial macrophage infiltration. A comprehensive review on MCP-1 and diabetes found that hyperglycemia increases the production of MCP-1 and that it is associated with many of the long-term complications in diabetes (Panee, 2012).

***High-sensitivity C-Reactive Protein (Hs-CRP).*** Hs-CRP is not a cytokine but is produced in the liver in response to inflammation (Wang et al., 2013). In prospective studies, CRP was associated with risk of T2DM (Wang et al., 2013). Sleep disturbance and shorter sleep duration also were associated with higher levels of CRP (Irwin et al., 2016).

***WBC.*** Higher levels within the normal range are observed in obese subjects with insulin resistance compared to obese subjects without it (Ryder et al., 2014). In addition, higher incidence of metabolic syndrome was observed when subjects in upper quartiles of total leukocyte count were compared to the lowest (Babio et al., 2013). Lymphocytes are also correlated with insulin levels (Ryder et al., 2014).

Each of the analytes IL-6, IL-8, TNF-α, and MCP-1, were run in duplicate, and in batches to provide a mean value. For values reported as below assay detection level, we used the assay threshold numeric value. In addition, if the duplicate values differed by greater than 20%, a mean of the two values was used. If one of the duplicates was reported as 0, the value from the other run was used.

**Statistical Analysis**

The supplemental outcomes for this study were not the primary outcomes of the parent trial and were not powered. Supplementary outcomes were exploratory. We used linear mixed models to analyze group differences following the intervention for each outcome variable. The model included interactions between time, diet and level of support. The model also controlled for the stratifying variables sex and baseline BMI (modeled as a continuous variable); and included the random intercept of participant. Each inflammatory marker was log transformed (used as the outcome variable) due to the skewed nature of their distributions. The residuals were also examined to test the assumptions of the linear mixed models. Due to residuals greater than

an absolute value of two, a sensitivity analysis was conducted to test the effects of these observations. The Bonferroni correction for multiple comparisons was used to protect from type 1 error due to the number of outcome measures being studied. While the parent trial examined the effects of varying levels of support, it was not pertinent to our research questions. Therefore, we collapsed the diet and level of support groups to diet group to increase power during comparison tests. Stata provides a post-estimation option called Margins to aid in understanding and interpreting model results. This option was used to provide the estimated marginal means (EMM) and to test the difference in differences. EMMs provide the estimated means based on the model at baseline and 4 months. The EMM values were used to calculate the change by diet group. The difference in difference compares the change observed in the VLC diet group to the change observed in the DASH diet group. Finally, we planned to analyze mediation using structural equation modeling if significant effects of diet on inflammation were observed. Mediation analyses seek to understand the mechanism behind observed relationships. Structural equation modeling is a statistical analysis technique used to analyze the structure of relationships, including the effects of mediation. We consulted a statistician for all statistical analyses. Stata version 16, College Station, TX, was used for all statistical analyses.

## Results

Recruitment occurred from January of 2019 through March of 2020. Results are from the 79 participants enrolled through October 2019 of which 58 had completed the intervention. Summary characteristics for all participants, and by diet group at baseline, are presented in Table 3.1. The sample was 64.56% female, 72.60% non-Hispanic White, 19.18% non-Hispanic Black, 2.74% Asian, 5.48% Hispanic ethnicity, and had a mean age of 58.84.

The parent study found that the VLC diet was more effective at decreasing BMI (VLC at baseline vs. at 4 months was 35.59 vs. 33.11 kg/m$^2$), compared to the DASH diet (EMM at baseline vs. at 4 months was 35.59 vs. 34.18 kg/m$^2$), difference in difference for VLC compared to DASH = -1.08 kg/m$^2$. The VLC diet was also more effective at decreasing HbA1c (VLC EMM at baseline vs. at 4 months was 6.08 vs. 5.72 %), compared to DASH (EMM at baseline vs. at 4 months was 6.08 vs. 6.01 %). The difference in difference for HbA1c in the VLC group compared to DASH is -.28%.

The following are the results from the present supplemental trial. A sensitivity analysis was completed based on the absolute value of the residuals. Dropping the observations with residuals greater than an absolute value of two did not alter significance testing for any of our outcomes. Table 3.2 presents results from the analyses on all observations, including observations with high residuals. EMM results for this supplemental trial at baseline and 4 months, difference within arm, and difference in difference are presented in Table 2. The VLC diet was more effective at reducing fasting insulin (VLC EMM at baseline vs. at 4 months was 19.81 vs. 14.00 µU/mL), compared to DASH (EMM at baseline vs. at 4 months was 19.11, vs. 17.16 µU/mL, p = .027). Similarly, VLC participants also experienced greater reductions in HOMA2-IR (EMM at baseline vs. 4 months was 2.62 vs. 1.85), compared to the DASH diet group (EMM at baseline vs. at 4 months was 2.55 vs. 2.29, p = .029). However, the effects on fasting insulin and HOMA2-IR were no longer significant when a Bonferroni adjustment for multiple comparisons was used. Participants in the VLC groups experienced a greater reduction in WBC (EMM at baseline vs. at 4 months was 6.32 vs. 5.98 K/uL) compared to the DASH diet groups (EMM at baseline vs. at 4 months was 5.87 vs. 6.24 K/uL, p = .004). This effect on WBC was the only outcome to remain significant after the Bonferroni adjustment for multiple

comparisons. The DASH diet group experienced a greater reduction in IL-6 (EMM at baseline vs. at 4 months was 2.15 vs. 1.69 pg/mL), compared to participants in the VLC group (EMM at baseline vs. at 4 months was 3.38 vs 3..69 pg/mL), and the difference in difference approached significance (VLC compared to DASH .77, p = .070). We did not observe significant differences between diet groups for the other inflammatory markers, before the Bonferroni-adjustment: hs-CRP (VLC EMM at baseline vs. at 4 months was 5.26 vs. 5.37), compared to the DASH diet group (EMM at baseline vs. at 4 months was 3.13 vs. 3.08 mg/dL), IL-8 (VLC EMM at baseline vs. at 4 months was = 7.13, 8.09; DASH EMM at baseline vs. at 4 months was 4.77 vs. 4.40 pg/mL ), TNF-α (VLC EMM at baseline vs. at 4 months was 4.68 vs. 5.44; DASH EMM at baseline vs. at 4 months was 4.01 vs. 4.73 pg/mL), MCP-1 (VLC EMM at baseline vs. at 4 months was 345.27 vs. 393.59; DASH EMM at baseline vs. at 4 months was 387.09 vs. 362.59 pg/mL). Figures 3.1-3.4 show the effects over time on outcomes by diet group. Figure 3.1 illustrates fasting insulin, Figure 3.2 illustrates HOMA2-IR, Figure 3.3 illustrates WBC, and Figure 3.4 illustrates IL-6. We were unable to test whether insulin mediated the effect between diet and inflammation due to the small sample size and limited effects observed on inflammatory markers.

## Discussion

The VLC diet was more effective than the DASH diet at reducing WBC, which held after the Bonferroni-adjustment. However, the DASH diet resulted in a greater decrease in IL-6, although this relationship was not statistically significant. We did not observe significant between-group changes in other inflammatory markers. The VLC diet produced greater reductions in fasting insulin and HOMA2-IR, although this relationship did not hold after the Bonferroni-adjustment for multiple comparisons.

This study was a pilot trial and not powered to test these examined effects, so further powered trials are warranted. In addition, the full recruitment of 120 subjects was not achieved. Additionally, the trial had a duration of 4 months, which may not have provided adequate time to observe an effect on inflammation. However, previous trials have observed effects on inflammation following interventions of 4 months or less (Al-Sarraj, Saadi, Calle, Volek, & Fernandez, 2009; Forsythe et al., 2008a). This may be due to the study population used. Some trials with effects observed within 4 months utilized participants with fewer comorbidities. For example, Forsythe et al. (2008a) decreased CRP, TNF-α, IL-6, IL-8 and MCP-1, in a sample of overweight men and women with dyslipidemia using a very low-carbohydrate diet for 12 weeks. That trial utilized in-person weekly visits with a Registered Dietitian (Forsythe et al., 2008a). Therefore, the method of education (in-person versus online), education level of the coach/educator, or health of the participants may be responsible for the differences observed. Additionally, Al-Sarraj et al. (2009) decreased CRP, TNF-α, ICAM-1, and MCP-1 at 6 weeks in a population with metabolic syndrome using a carbohydrate-restricted diet of 20-25% carbohydrate. That trial also used Registered Dieticians and in-person visits. Previous research suggests that longer trials may be needed to observe effects on inflammatory markers in a sample of adults with diabetes. For example, in Hallberg et al. (2018) hs-CRP was measured at 70 days and one year in their trial utilizing a very-low carbohydrate diet intervention to the standard of care for one year. Differences were observed in the ability of the intervention to produce an effect based on the intensity of the intervention. At 70 days, significant decreases in hs-CRP were not observed in any group, although effects were observed in all arms at one year (Hallberg et al., 2018). The present trial was an online trial, designed to be scalable and cost-effective. Therefore, an important next step would be to investigate whether such a low-resource trial may

provide similar effects on inflammation over a longer time period. Additionally, the three trials outlined above had good dietary adherence. As the parent trial is ongoing, dietary adherence data are not yet available, but in the future may yield insight into the results obtained from a lower resource trial in overweight or obese participants with prediabetes or T2DM and hypertension.

Additionally, the VLC diet tended to produce larger reductions in fasting insulin and insulin resistance compared to the DASH diet, which was consistent with the hypothesis due to the decreased carbohydrate load of the VLC diet. However, there were inconsistent results when examining the effect of diet on inflammation. The VLC arm was more effective at significantly decreasing WBC, while the DASH arm appeared more effective at decreasing IL-6, although the difference did not reach statistical significance. However, IL-6 is also produced in skeletal muscle during exercise (Pedersen, Steensberg, & Schjerling, 2001), so potential differences in exercise may account for observed differences. The DASH diet provided a stronger emphasis on consuming fruits and vegetables, an effective approach at decreasing inflammation (Mahoney & Loprinzi, 2014). The DASH diet also promoted the intake of magnesium, which may also decrease inflammation (Chacko et al., 2010). The VLC diet emphasized limiting vegetable consumption to low-carbohydrate vegetables but did not provide specific recommendations to consume a minimum number of servings daily. It may be more effective to promote a minimum consumption of low-carbohydrate vegetables in the VLC diet as well to ensure that certain anti-inflammatory nutrients such as magnesium are consumed in adequate amounts. A diet that combines the more effective attributes of each diet may be more effective at simultaneously decreasing fasting insulin, insulin resistance, and inflammation.

A weight loss of 10% may be needed to decrease inflammation (Forsythe, Wallace, & Livingstone, 2008b). We hypothesized that the reduction of insulin would mediate a reduction in

inflammation, separate from the effects of weight loss. However, due to the limited sample size and short duration, we were unable to test this hypothesis. As the VLC group experienced a greater reduction in fasting insulin, and WBC was the only inflammatory marker to experience a significant reduction following the Bonferroni-adjustment, this relationship needs further investigation. Future trials would benefit from a larger sample and longer duration to test the effect of insulin in mediating this relationship between diet and inflammation.

The limitations in the present study reflect the limitations of pilot studies in general. We had a small sample size and a treatment duration limited to 4 months. Additionally, we experienced problems with recruitment due to the requirement for a systolic blood pressure above 130 mmHg (with or without current use of antihypertensive medications). While this minimum systolic blood pressure was used to stay consistent with the current American Heart Association goals for blood pressure control (Whelton et al., 2017), 97 participants were excluded due to this requirement. Future trials should alter this exclusion to aid in recruitment.

The VLC diet was more effective at decreasing WBC, which remained significant after the Bonferroni adjustment. However, the DASH group tended to be more effective at decreasing IL-6, although this was not statistically significant. The VLC diet tended to be more effective at decreasing both fasting insulin and insulin resistance. Future trials of longer duration and larger sample sizes are needed to evaluate the effectiveness of these diets at decreasing insulin and inflammation, and whether insulin is partially mediating this relationship.

Table 3.1

Summary Characteristics of Participants at Baseline

| Variable | All | VLC | DASH |
|---|---|---|---|
| Gender, N (%) | | | |
| Female | 51 (64.56) | 24 (63.16) | 27 (65.85) |
| Male | 28 (35.44) | 14 (36.84) | 14 (34.15) |
| Race/Ethnicity, N (%) | | | |
| White | 53 (72.60) | 26 (76.47) | 27 (69.23) |
| Black | 14 (19.18) | 6 (17.65) | 8 (20.51) |
| Asian | 2 (2.74) | 0 (0) | 2 (5.13) |
| Hispanic | 4 (5.48) | 2 (5.88) | 2 (5.13) |
| Age years, Mean (SD) | 58.84 (7.95) | 57.61 (9.17) | 59.98 (6.53) |
| Baseline BMI $kg/m^2$, Mean (SD) | 35.88 (5.58) | 35.48 (5.42) | 36.24 (5.76) |
| Baseline Glucose mg/dL, Median (IQR) | 109.00 (101.00, 115.00) | 106.50 (96.00, 113.00) | 111.00 (102.00, 118.00) |
| Baseline HbA1c%, Median (IQR) | 6.00 (5.80, 6.30) | 6.00 (5.80, 6.30) | 6.00 (5.70, 6.30) |

Abbreviations: N – number, SD – standard deviation, IQR – interquartile range, White – non-Hispanic White, Black – non-Hispanic Black
Means and SD used for normal distributions, Median and IQR used for skewed distributions

Table 3.2

Comparison of DASH Diet to VLC Diet Change Relative to Baseline

| Outcome Variable | Baseline EMM (SE) | 4 months EMM (SE) | Difference Within Diet Arm | Difference in Difference | Difference in Difference P-value |
|---|---|---|---|---|---|
| Fasting Insulin µU/mL | | | | -3.86 | .027 |
| VLC | 19.81 (1.47) | 14.00 (1.13) | -5.81 | | |
| DASH | 19.11 (1.35) | 17.16 (1.34) | -1.95 | | |
| HOMA2-IR | | | | -0.51 | .029 |
| VLC | 2.62 (0.19) | 1.85 (0.15) | -0.77 | | |
| DASH | 2.55 (0.18) | 2.29 (0.18) | -0.26 | | |
| Hs-CRP mg/dL | | | | 0.16 | .864 |
| VLC | 5.26 (0.80) | 5.37 (0.89) | 0.11 | | |
| DASH | 3.13 (0.45) | 3.08 (0.50) | -0.05 | | |
| IL-6 pg/mL | | | | 0.77 | .070 |
| VLC | 3.38 (1.03) | 3.69 (1.13) | 0.31 | | |
| DASH | 2.15 (0.64) | 1.69 (0.52) | -0.46 | | |
| IL-8 pg/mL | | | | 1.32 | .090 |
| VLC | 7.13 (1.24) | 8.09 (1.45) | 0.96 | | |
| DASH | 4.77 (0.84) | 4.40 (0.78) | -0.37 | | |
| TNF-α pg/mL | | | | 0.04 | .94 |
| VLC | 4.68 (0.40) | 5.44 (0.51) | 0.76 | | |
| DASH | 4.01 (0.33) | 4.73 (0.44) | 0.72 | | |
| MCP-1 pg/mL | | | | 72.82 | .092 |
| VLC | 345.27 (26.96) | 393.59 (33.52) | 48.32 | | |
| DASH | 387.09 (29.07) | 362.59 (30.83) | -24.5 | | |
| WBC K/uL | | | | -0.72 | .004* |
| VLC | 6.32 (0.25) | 5.98 (0.22) | -.33 | | |
| DASH | 5.87 (0.23) | 6.24 (0.23) | .37 | | |

EMM – estimated marginal mean, SE – standard error
* significance held after Bonferroni adjustment for multiple comparisons

Figure 3.1

Change in Fasting Insulin over 4-Month Intervention by Diet Group

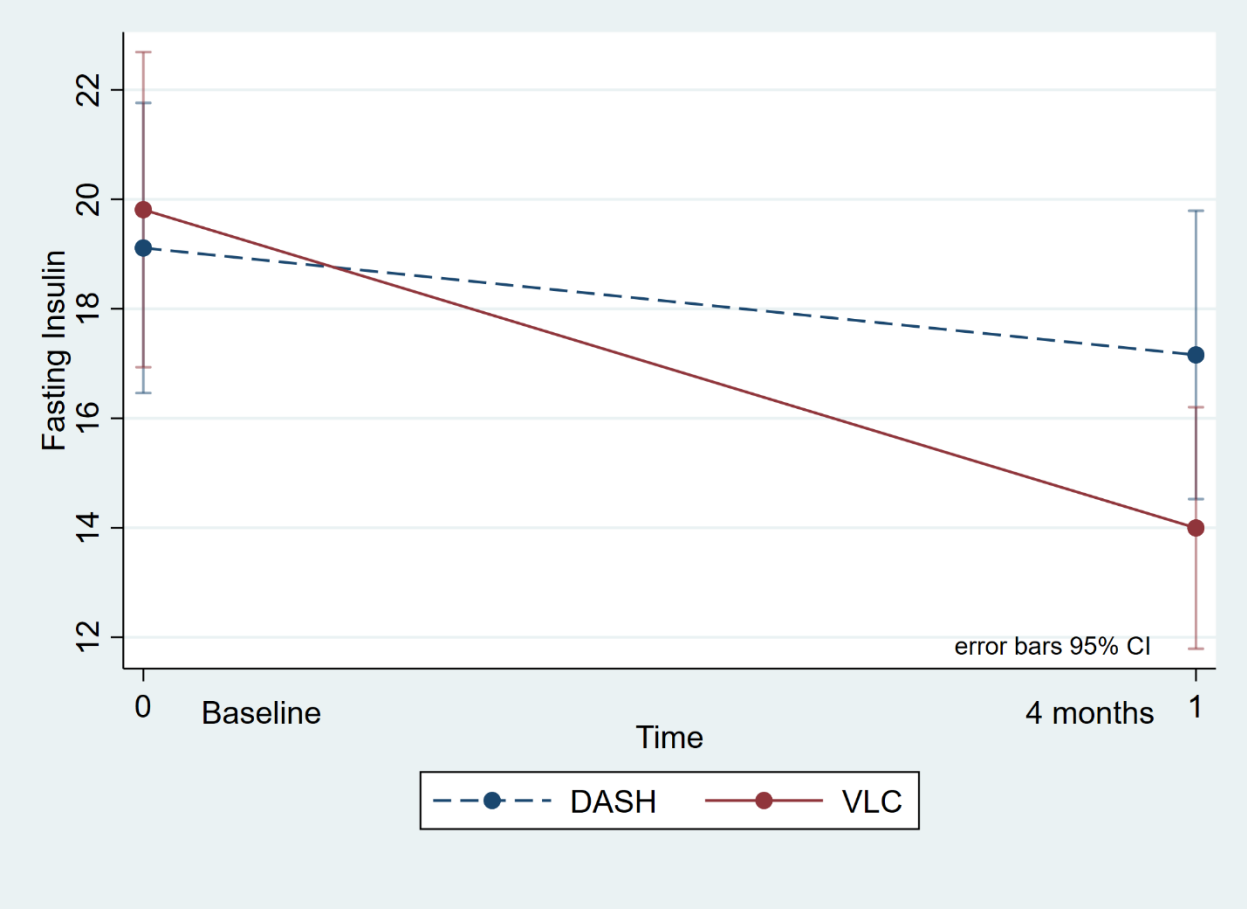


Figure 3.2

Change in HOMA2-IR over 4-Month Intervention by Diet Group

Figure 3.3

Change in WBC over 4-Month Intervention by Diet Group

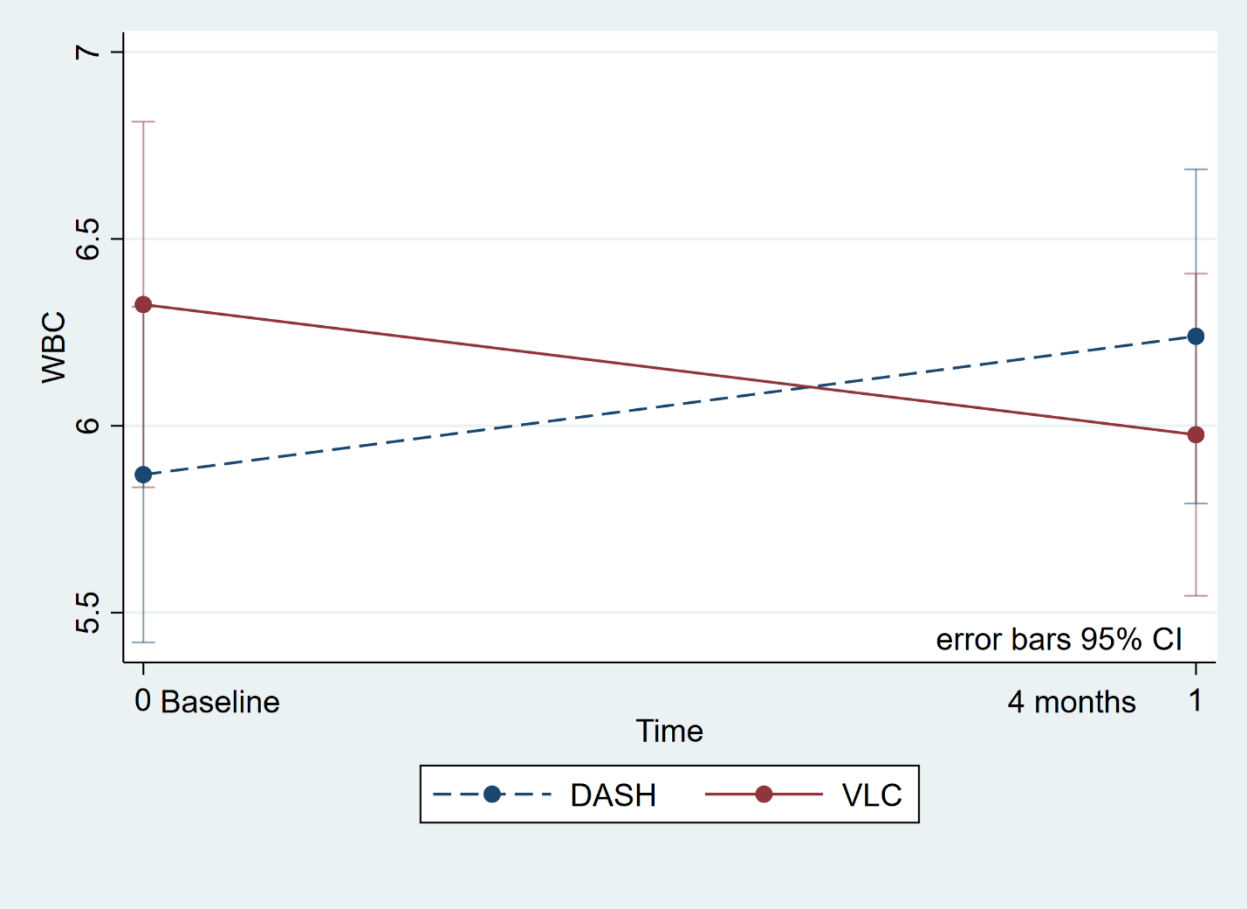
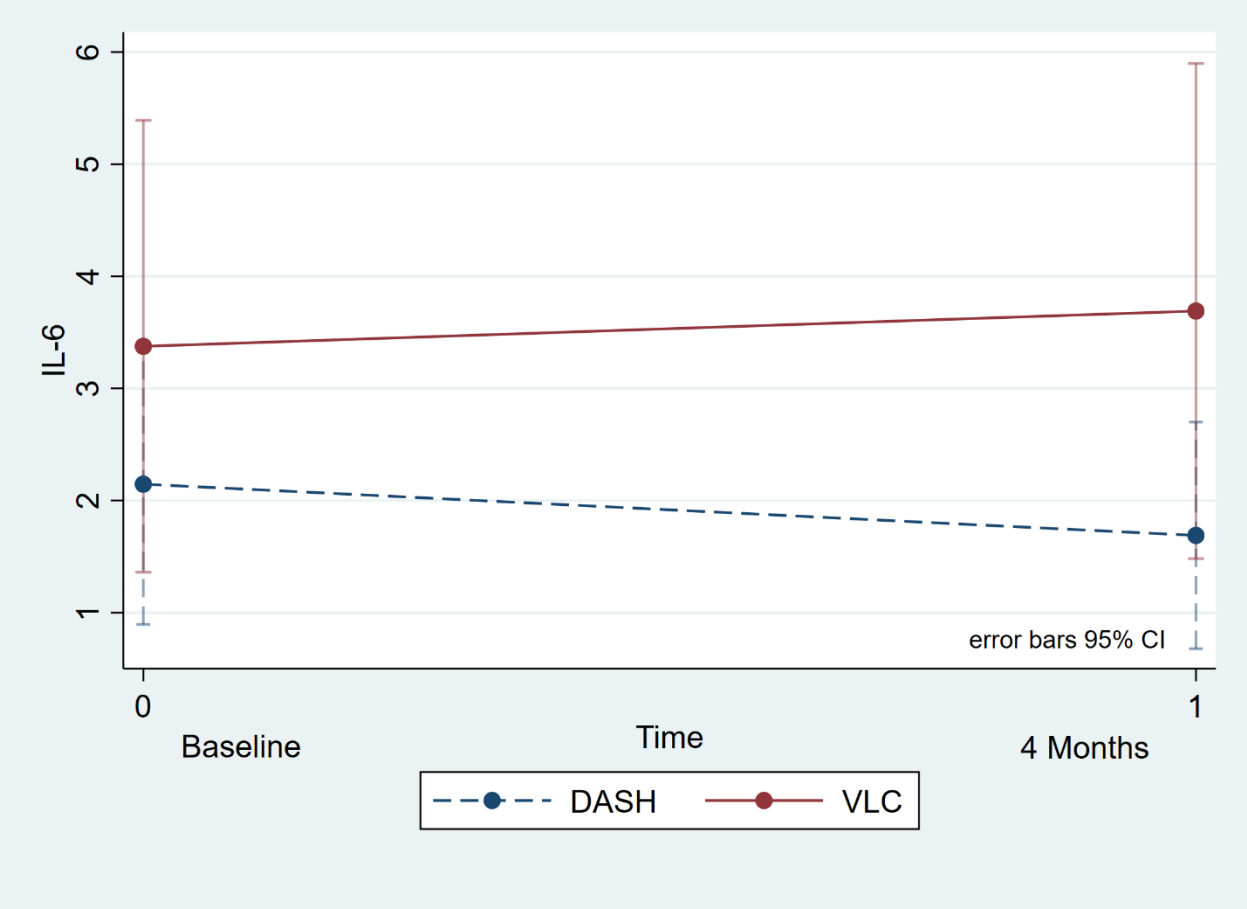


Figure 3.4

Change in IL-6 Over 4-Month Intervention by Diet Group



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# Chapter Six

## Discussion

In the United States, there are over 100 million adults diagnosed with prediabetes or type 2 diabetes (T2DM) (U.S. Department of Health and Human Services, 2020). Hyperglycemia and insulin resistance are key defects in T2DM (Wilcox, 2005). Inflammation is involved in the pathogenesis (Donath & Shoelson, 2011), progression (Rehman et al., 2017), and long-term complications of T2DM (Panee, 2012). However, current recommendations do not include goals for decreasing inflammation (American Diabetes Association, 2020; Garber et al., 2020). The pathogenesis of inflammation in prediabetes and T2DM is likely multifactorial and may vary based on stage of disease progression. Hyperglycemia and visceral adipose tissue, which are both common in individuals with T2DM, are both proinflammatory (Jafar, Edriss, & Nugent, 2016; Makki, Froguel, & Wolowczuk, 2013). However, previous research also suggests that hyperinsulinemia is associated with the development of inflammation (Pedersen et al., 2015). Diets capable of simultaneously decreasing insulin and glucose levels may improve the outcomes for those diagnosed with, and at risk for, T2DM. Overall, this dissertation has sought to increase our understanding of the effect of diet on inflammation, and the role of insulin as a mediator in this pathway.

## Results

### **Aim 1** - ***Association Between Fasting Insulin and High-Sensitivity C-Reactive Protein Among Adults Without Diabetes: NHANES 2005-2010***

Examine the association between fasting insulin and high-sensitivity C-reactive protein (hs-CRP), a marker of inflammation, using the National Health and Nutrition Examination Survey (NHANES), 2005-2010. I hypothesized the existence of a significant positive association between fasting insulin and hs-CRP.

**Findings**. This analysis was completed on a sample of adults without a diagnosis of diabetes. A sample without diabetes was selected due to the effects of hyperglycemia, beta cell failure, and glucose-lowering medications effect on insulin levels that would occur in a sample with diabetes. After adjusting for age, race, gender, smoking status, physical activity, and poverty-income ratio, fasting insulin level was significantly associated with high-risk hs-CRP compared to average-risk hs-CRP (RRR 1.07, $p < .001$). While adjusting for the same covariates and waist circumference, the RRR was reduced to 1.02, $p = .002$. Thus, the inclusion of waist circumference as a covariate attenuated the association between fasting insulin and high-risk hs-CRP by 68%, indicating some role of visceral adipose tissue in mediating the relationship between fasting insulin and hs-CRP. We also found a significant association between fasting insulin and low-risk hs-CRP compared to average risk hs-CRP in both the reduced adjusted model ($\beta = 0.91$, $p < .001$) and the full adjusted model ($\beta = 0.91$, $p < .001$). The relationship between fasting insulin and low-risk hs-CRP was attenuated 69% between the full and the reduced models.

**Conclusions.** There was a significant association between fasting insulin and hs-CRP which was only partially mediated by waist circumference. Treatment approaches that simultaneously decrease insulin while achieving glycemic control may decrease inflammation.

**Aim 2 –** ***Association Between Net Carbohydrate and Sugar Intake on Inflammation and Hemoglobin A1c in Adults with Diabetes***

Examine the association between net carbohydrate and sugar intake on white blood cell count at the upper end of the normal range, another indicator of inflammation, and HbA1c, using the National Health and Nutrition Examination Survey, 2011-2016. I hypothesized the existence of a significant positive association between net carbohydrate and sugar intake and white blood cell count (WBC) and on hemoglobin A1c (HbA1c).

**Findings.** This analysis was completed in a sample of adults with a diagnosis of diabetes and provided national estimates. Most participants were using glucose-lowering medications, 61.71% oral medications and 19.12% were using insulin. The first two models examined the effect of diet (net carbohydrate or total sugar) on WBC. After adjusting for age, sex, race, poverty-income ratio, smoking status, physical activity, use of cholesterol medication, and waist circumference, there was a moderate but significant association between net carbohydrate intake and WBC ($\beta$ = 0.001, p = .047). However, after adjusting for the same confounders, there was no association between total sugar intake and WBC ($\beta$ = 0.000, p = .838). The next two models examined the effect of diet (net carbohydrate versus total sugar) on HbA1c. In the adjusted model which controlled for age, sex, race, poverty-income ratio, smoking status, physical activity and waist circumference, neither total net carbohydrates ($\beta$ = 0.001, p = .311) nor total sugar ($\beta$ = -0.000, p = .815), were associated with HbA1c.

**Conclusions.** There was a significant association between net carbohydrate intake and WBC concentration at the higher end of the normal range, which suggests that decreasing carbohydrate load may decrease inflammation.

**Aim 3 –** ***Effects of a Very Low-Carbohydrate Diet Versus a Dietary Approaches to Stop Hypertension Diet on Markers of Inflammation***

(a) Compare the effects of a very low-carbohydrate diet (VLC) and a Dietary Approaches to Stop Hypertension diet (DASH), on fasting insulin, insulin resistance, and inflammatory markers. Based on suggestive evidence from previously conducted literature (Al-Sarraj, Saadi, Calle, Volek, & Fernandez, 2009; Asemi, Samimi, Tabassi, Sabihi, & Esmaillzadeh, 2013; Hallberg et al., 2018; Jonasson, Guldbrand, Lundberg, & Nystrom, 2014; Shirani, Salehi-Abargouei, & Azadbakht, 2013; Steckhan et al., 2016), I hypothesized the existence of clinically significant improvements insulin, insulin resistance, and inflammatory markers for participants assigned to a VLC diet, compared to lesser improvements for those assigned to the DASH diet arm. (b) Examine whether VLC- or DASH-associated changes in fasting insulin mediate the effect of diet on inflammatory markers IL-6, IL-8, TNF-α, MCP-1, hs-CRP, or WBC count. I hypothesized the existence of a significant positive association between fasting insulin and dietary effect on inflammation.

**Findings 3a.** This study was carried out in a sample of overweight or obese adults with hypertension and prediabetes or T2DM. Overall, the VLC diet tended to be more effective at reducing fasting insulin (VLC EMM at baseline vs. at 4 months was 19.81 vs. 14.00 µU/mL), compared to DASH (EMM at baseline vs. at 4 months was 19.11 vs. 17.16 µU/mL, $p = .027$) , and HOMA2-IR (VLC EMM at baseline vs. at 4 months was 2.62 vs. 1.85), compared to the DASH diet group (EMM at baseline vs. at 4 months was 2.55 vs. 2.29, $p = .029$). However, the effects on fasting insulin and HOMA2-IR were no longer significant after a Bonferroni adjustment for multiple comparisons. The VLC group experienced greater and significant reductions in the inflammatory marker of WBC counts at the upper end of the normal range (EMM at baseline vs. at 4 months was 6.32 vs. 5.98 K/uL) compared to the DASH diet group (EMM at baseline vs. at 4 months was 5.87 vs. 6.24 K/uL, $p = .004$), which remained after the

Bonferroni adjustment for multiple comparisons. The DASH diet group experienced greater reductions in IL-6 (EMM at baseline vs. at 4 months was 2.15 vs. 1.69 pg/mL), compared to participants in the VLC group (EMM at baseline vs. at 4 months was 3.38 vs. 3.69 pg/mL), which approached significance (VLC compared to DASH .77, p = .070). Significant differences between diet groups were not observed for the other inflammatory markers before the Bonferroni-adjustment. The direct inflammatory measure of WBC counts was the only outcome to remain significant after the Bonferroni adjustment for multiple comparisons.

**Conclusions.** The VLC diet was more effective at decreasing WBC and tended to be more effective at decreasing fasting insulin and insulin resistance. However, the DASH diet tended to be more effective at decreasing IL-6.

**Findings 3b.** We were unable to test whether insulin mediated the effect between diet and inflammation due to the small sample size and limited effects observed on inflammatory markers.

## Limitations

There were several limitations to these studies. Two of the trials utilized cross-sectional NHANES data, which do not allow the inference of cause and effect. Additionally, the study examining the effect of net carbohydrate and sugar intake (Aim 2) used aggregate dietary data. This prevented us from distinguishing between added sugar and naturally occurring sugar, and between whole grains versus highly refined carbohydrates. Previous research suggests that added sugar may be more proinflammatory than naturally present sugar, and that glycemic index or glycemic load may impact the inflammatory response. Therefore, this was a limitation in Aim 2 (Aeberli et al., 2011; DiNicolantonio, Mehta, Onkaramurthy, & O'Keefe, 2018; O'Keefe, Gheewala, & O'Keefe, 2008).

In the final Aim 3 study we were limited by a small sample size as we experienced problems with recruitment due to the requirement for a systolic blood pressure above 130 mmHg (with or without current use of antihypertensive medications). Moreover, this study had a treatment duration of just four months. While effects on inflammation have been observed in 4-month trials in populations with fewer comorbidities (Al-Sarraj et al., 2009; Forsythe et al., 2008), a longer duration may be needed in individuals diagnosed with T2DM (Hallberg et al., 2018). The study used in Aim 3 was not powered to test effects and the outcomes analyzed were exploratory.

## Clinical and Policy Implications

Current standards of care for the treatment of T2DM do not provide recommendations for decreasing inflammation (American Diabetes Association, 2020; Garber et al., 2020). However, there is evidence that inflammation is involved in the long-term complications of T2DM. This has important implications, both economic and related to quality of life. In 2017, direct and indirect costs of diagnosed diabetes was 327 billion (U.S. Department of Health and Human Services, 2020). Cost-analyses of diabetes suggest that the majority of spending related to diabetes care was for the treatment of advanced complications in older adults (Herman, 2013). A breakdown of these costs found that hospital inpatient visits, nursing homes, hospice care, home health care, and prescriptions medications for complications of diabetes accounted for almost three-quarters of health care expenditures (Herman, 2013).

Additionally, the most recent report from the CDC on the current state of diabetes in the US found that 50% of adults with diabetes had an A1c above 7% (U.S. Department of Health and Human Services, 2020). Current treatment recommendations from the American Diabetes Association (ADA) recommend a goal HbA1c of less than 7%. Therefore, current treatment

approaches are not effective for approximately half of US adults with diabetes. The cost of medications may be one barrier to treatment. The ADA issued a report on the economic impact of diabetes in 2017 (Yang et al., 2018). The cost of medications has increased by 45% from 2012 to 2017, after adjusting for inflation (Yang et al., 2018). Results from the National Health Interview Survey suggest cost is a barrier to Americans receiving the medications necessary to control their blood sugar (Knight, Probst, Liese, Sercye, & Jones, 2016). The survey asked four questions about altered medication use related to cost and 18.9% of participants with diabetes responded yes to at least one question (Knight et al., 2016). Reducing the carbohydrate load or postprandial glycemic excursions would likely decrease the need for glucose-lowering medications. Trials have documented success of needing lower doses or eliminating the use of glucose-lowering medications altogether, including insulin injections by decreasing the carbohydrate load (Hallberg et al., 2018). This may prove a more viable option for those who are unable to pay for medications.

To address this issue, we need to address the deficiencies in current recommendations. Addressing chronic inflammation and setting goals to control it would be an important first step. Secondly, effective diet interventions capable of simultaneously decreasing glucose levels and inflammation need to be investigated and disseminated.

## Conclusions

The current treatment recommendations for T2DM do not address the benefit of decreasing inflammation (American Diabetes Association, 2020; Garber et al., 2020). However, there is evidence, including from our study, that prevention and treatment efforts may be improved if this is addressed. These results suggest that treatment approaches that simultaneously decrease insulin levels while achieving glycemic control may provide additional

anti-inflammatory effects, and therefore may improve long-term outcomes for adults with T2DM. As carbohydrates are the predominant macronutrient responsible for postprandial glucose excursions and insulin response (Evert et al., 2019), reducing carbohydrate load may improve glycemic control while simultaneously decreasing inflammation.

## Future Research

Future trials of longer duration and larger samples are needed to evaluate the effectiveness of diet at decreasing insulin and inflammation, and whether insulin is partially mediating this relationship. Additionally, understanding how postprandial glucose excursions impact inflammation both in the short- and long-term will increase our understanding of the effect so that dietary guidelines may provide more nuanced recommendations

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